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THE BIOLOGY AND PATHOLOGY OF

Dwarfmistletoe

Arceuthobium campylopodum f. abietinum

Parasitizing True Firs

(*Abies spp.*)

In California

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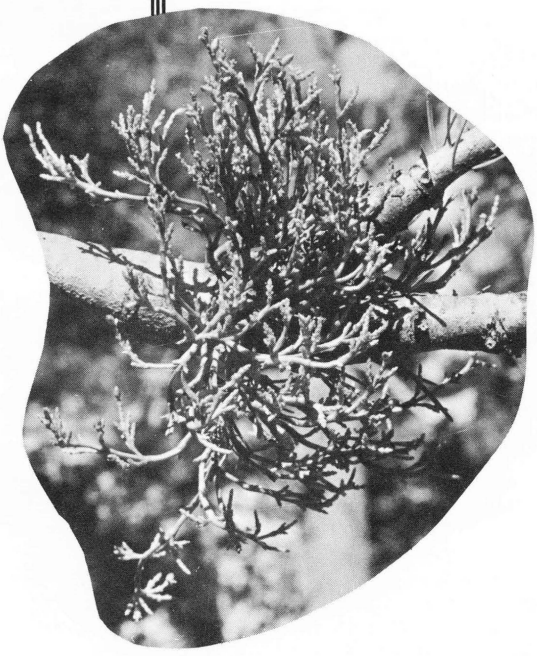

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Parasitizing True Firs

(*Abies spp.*)

In California

By R. F. Scharpf, *Plant Pathologist, Pacific Southwest Forest and Range Experiment Station, Berkeley, Calif.*, and J. R. Parmeter, Jr., *Associate Professor, Department of Plant Pathology, University of California, Berkeley*

INTRODUCTION

Dwarfmistletoes (*Arceuthobium* spp.), considered the most important disease problem in California forests, cause extensive damage to coniferous timber species throughout the West. Reduction of the damage depends on adequate knowledge of the biology and pathology of these parasites. Much needed information is lacking, and many points of dispute remain concerning the behavior of dwarfmistletoes. Information about the parasites on true firs (*Abies* spp.) is particularly meager, for most studies of dwarfmistletoes have been made on other timber species.

Forest disease surveys conducted by the U.S. Forest Service have shown that about 30 percent of the white fir (*Abies concolor* (Gord. & Glend.) Lindl.) stands and about 40 percent of the red fir (*A. magnifica*-A. Murr.) stands are infected with dwarfmistletoe (California Forest Pest Control Action Council 1961). Since true firs are heavily infected and constitute about one-fourth of the commercial timber volume in California, information is urgently needed to provide a basis for controlling losses and directing future management of infected fir stands. Consequently, studies were undertaken to (a) collect information on the behavior of dwarfmistletoes on red fir and white fir, (b) compare this behavior with that of dwarfmistletoes on other hosts, (c) help clarify some of the disputed aspects of the biology and pathology of dwarfmistletoes, and (d) develop guidelines for use by forest managers in planning control projects.

In this report, the host-specialized members of *Arceuthobium campylopodum* Engelm. f. *abietinum* (Engelm.) Gill (Parmeter et al. 1960) are distinguished by referring to the particular host on which they occur; for example, red fir dwarfmistletoe, white fir dwarfmistletoe. This simplified naming system is used merely to eliminate any doubt as to host-parasite affinities.

STUDY AREAS

The studies reported here were conducted on two areas in California, both having natural infection of firs by dwarfmistletoe.

Pinecrest, Stanislaus National Forest.—This area lies in the mixed conifer zone of the west slope of the central Sierra Nevada. In general, white fir occurs in mixed stands with ponderosa pine (*Pinus ponderosa* Laws.), Jeffrey pine (*P. jeffreyi* Grev. & Balf.), sugar pine (*P. lambertiana* Dougl.), and incense-cedar (*Libocedrus decurrens* Torr.). On the other hand, red fir is most prevalent in mixture with white fir or at higher elevations as pure stands. Firs used in the study ranged in elevation from about 5,000 to 7,500 feet.

Precipitation averages about 36 inches a year and occurs mainly as snow. Summers are dry with the exception of occasional thunderstorms. Warm summers prevail with temperatures ranging from about 40° to 95° F. Winter temperatures range from about −10° to about 60° F.

Latour State Forest.—The area is located on the west slope of the Cascades about 15 miles northwest of Mt. Lassen. White fir occurs in mixed stands with the species mentioned above and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Red fir in the area occurs in pure stands or is associated with white fir, lodgepole pine (*Pinus contorta* Dougl. ex Loud.), and western white pine (*P. monticola* Dougl.). Firs studied on the area occurred at elevations of about 4,500 to 6,000 feet.

Although precise climatological data are not available for the areas, precipitation averages about 50 inches a year, most of which occurs as snow. Daily temperatures average about 30° F. in January and about 65° F. in July. Summer thunderstorms accompanied by some precipitation are common.

FLOWERING AND SEED DISCHARGE

Information on the period and duration of dwarfmistletoe flowering is scant. Gill (1935) reported that all but one North American species bloom in the spring; *Arceuthobium campylopodum* blooms in August and September. However, Peirce (1905) stated that *A. campylopodum* on Monterey pine (*Pinus radiata* D. Don) flowers from September to January. In Europe, the height of the flowering period of *A. oxycedri* (DC.) Bieb. is in September and October (Heinricher 1915a, 1915c).

Information on fruit maturation and seed discharge of dwarfmistletoes is also meager. However, the period of seed discharge varies among the different species and forms of the parasite in different areas. In his comprehensive study of the genus, Gill (1935) reported that fruits of all the North American species mature in autumn; as a rule, the seeds are discharged by the end of October, but, under mild weather conditions the fruits may persist well into the winter. Roth (1959) reported that *Arceuthobium campylopodum* on ponderosa pine in Oregon discharged seed during September and October, whereas Dowding (1929) found that *A. americanum* Nutt. ex Engelm. on lodgepole pine in Alberta discharged seeds during the relatively short period between September 10 and 20. Seed discharge of *A. vaginatum* (Willd.) Presl. on ponderosa pine in Arizona and New Mexico occurred during July and August with maximum discharge the last week of July and the first 2 weeks of August (Hawksworth 1961). *A. campylopodum* on Monterey pine growing under mild conditions along the coast of California discharged seeds as late as December (Peirce 1905), as did *A. oxycedri* in Europe (Heinricher 1915b).

These reports of flowering and seed discharge emphasize differences both in time and duration of flowering and fruiting among the different species and forms of *Arceuthobium* in different areas. Therefore, investigations were made on the time, duration, and annual variation in flowering and seed discharge of dwarfmistletoe on true firs in California.

Methods

Observations on the flowering period of dwarfmistletoes on red and white fir were made at Pinecrest over the years 1961 through 1963. Observations on seed discharge in the same area extended over the years 1958 through 1963.

Flowering was considered to extend from the first appearance of opened staminate flowers (fig. 1) through the time when flowers were either dried up or no longer on the plant. The period of seed discharge was considered to extend from the first sign of seed discharge when plants were gently shaken until fruit (fig. 2) were no longer observed on shoots. Since it was not possible to determine exactly the duration of flowering and fruiting, the beginning and end of the flowering and seed discharge periods were estimated to the nearest week from observations made at about weekly intervals throughout the summer and fall.

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Figure 1.—Staminate flowers of dwarfmistletoe on red fir.

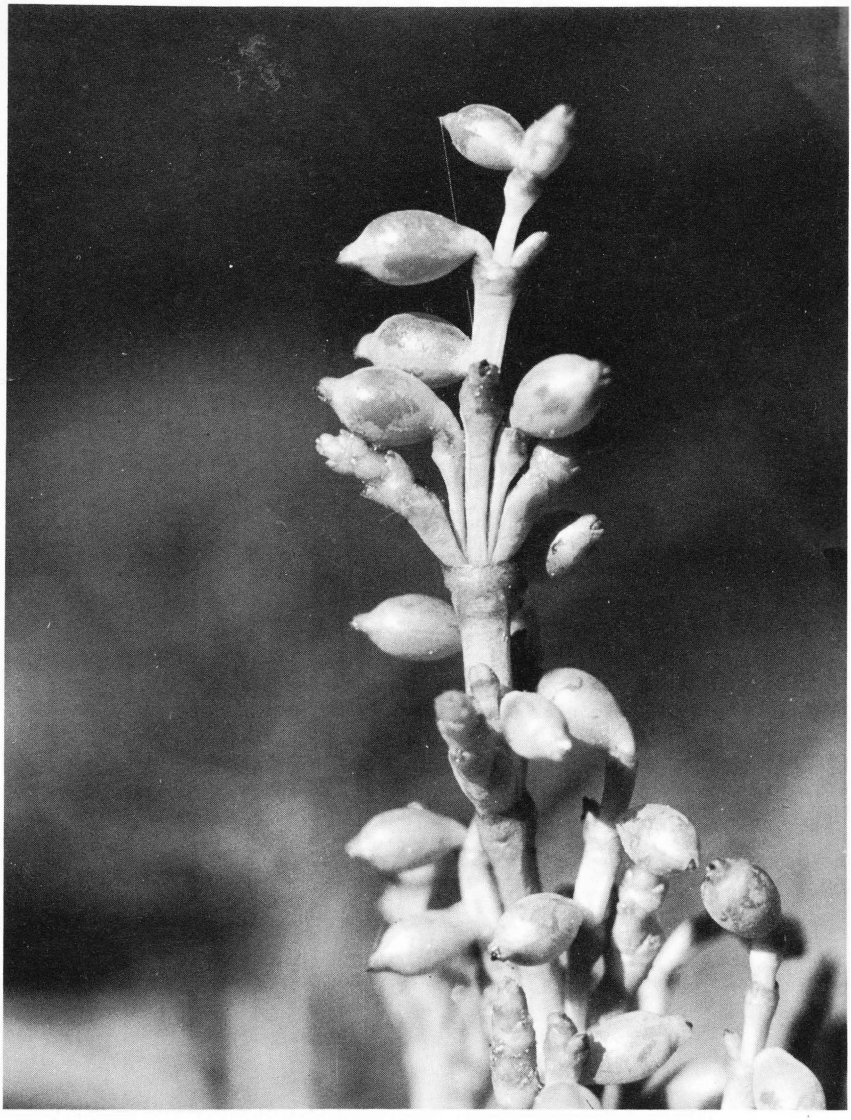

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Figure 2.—**Mature fruit of dwarfmistletoe on red fir.**

Results

In general, the pattern of flowering was similar for both the red fir and white fir dwarfmistletoes. In a given area certain plants bloomed earlier than others, and even on a single plant the flowers usually opened at different times. Also on certain plants pollen sacs of some open flowers were intact, whereas on others they were broken and pollen was exposed. Coincident with the opening of male flowers, female flowers were observed to exude droplets of nectar (fig. 3) and were presumed receptive to fertilization.

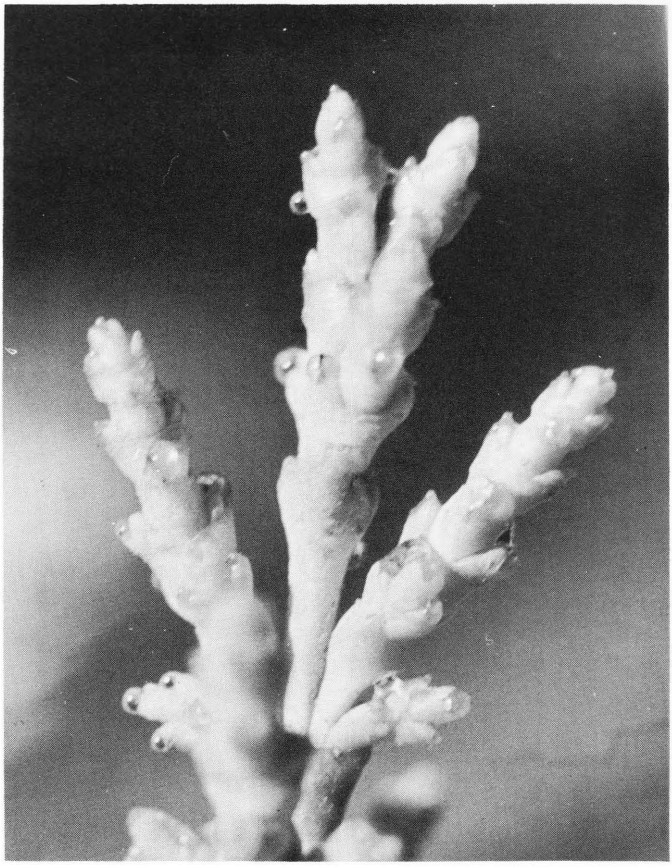

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Figure 3.—**Nectar exudation from pistillate flower of dwarfmistletoe on red fir.**

The flowering of these two dwarfmistletoes was quite comparable (fig. 4). The only difference was that the dwarfmistletoe on red fir, occurring at higher elevations, appeared to flower slightly earlier than the one on white fir.

The duration of flowering was also similar for both dwarfmistletoes over the 3 years observed. Nevertheless, rather marked differences were noted for the time of flowering in different years. For example, in 1962 the flowering period was about 1 to 2 weeks later and in 1963 about 4 weeks later than in 1961.

The pattern of seed discharge was similar for both fir dwarfmistletoes. Certain plants in a given area began discharge somewhat earlier in the season than others. Most of these plants were on the outer parts of branches or on parts of the tree exposed to direct sunlight. Seed discharge was frequently delayed for plants that were heavily shaded. Generally, in a particular area, the majority of the dwarfmistletoe plants began discharge at approximately the same time. The duration of discharge for individual plants appeared to depend on the size of the clump of fruit. Plants bearing only a few shoots with scattered fruit

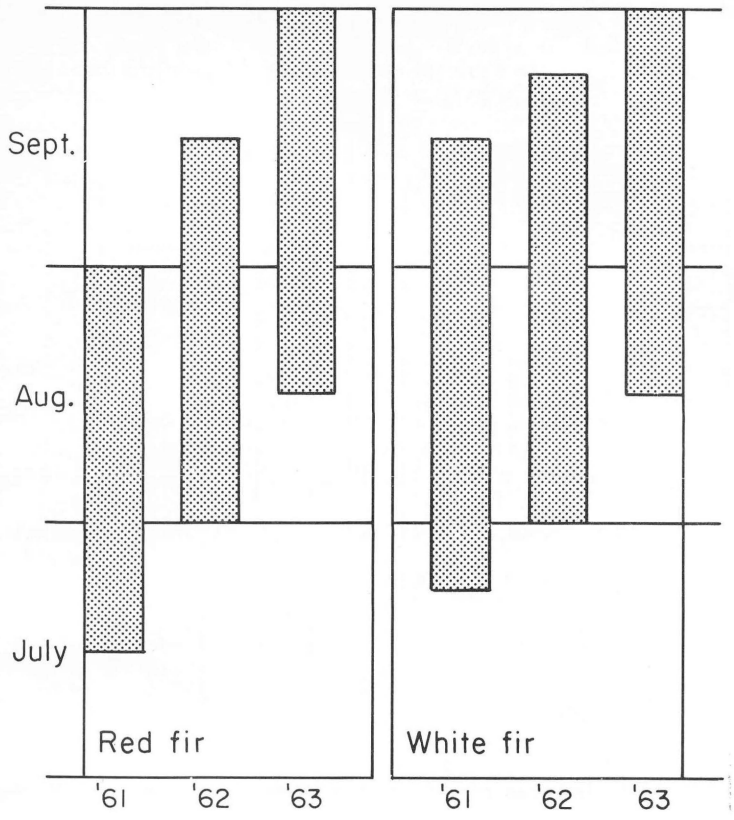

Figure 4.—**Flowering periods of dwarfmistletoe on red and white firs.**

discharged seeds over a relatively short period, whereas plants bearing large masses of fruit in densely packed clusters discharged seeds over an extended period. On these plants, the fruit in the outer part of the clusters matured and discharged seeds before the fruit near the center.

Nearly all seeds of dwarfmistletoe on firs were discharged during September and October (fig. 5). Except in 1962 and 1963, no marked differences in the period or duration of discharge were observed over the 6-year period. Dwarfmistletoe on white fir generally began discharge of seeds about a week later than the one on red fir, but the duration of discharge was about the same.

Discussion

The period of flowering and seed discharge of dwarfmistletoe in firs in California corresponded closely to that reported of other forms of *Arceuthobium campylopodum* in the mountainous regions of western North America (Gill 1935, Roth 1959).

The year-to-year variations of 1 to 4 weeks in beginning flowering and seed discharge probably resulted from differences in yearly climatic

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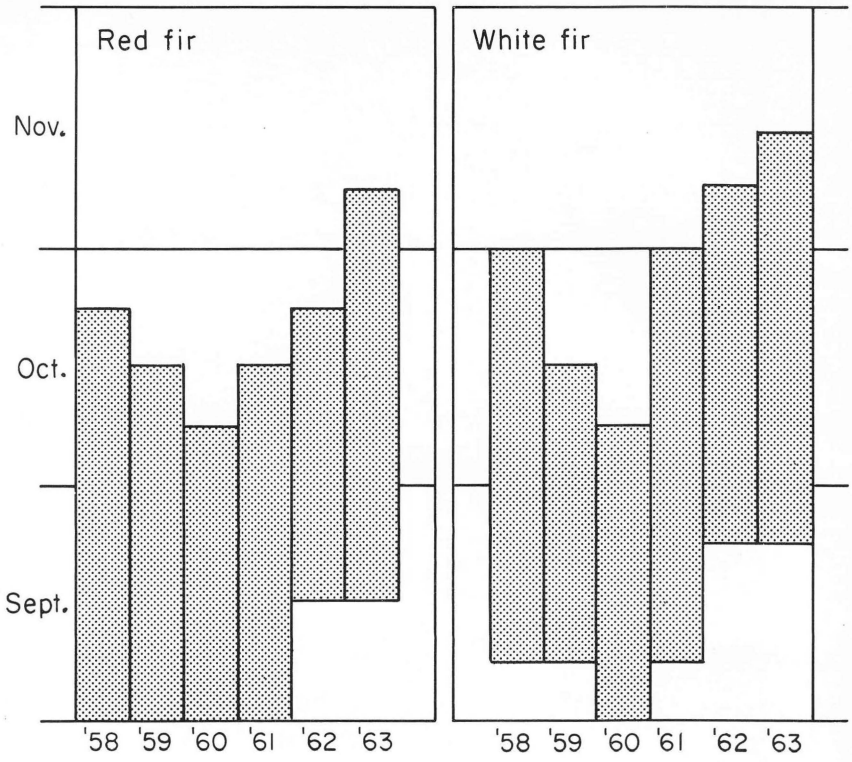

Figure 5.—**Seed discharge period of dwarfmistletoe on red and white firs.**

conditions. Somewhat more extreme climatic conditions prevalent in the higher elevation red fir stands likely accounted for slightly earlier flowering and seed discharge of the red fir dwarfmistletoe.

SEED DEPOSITION AND RETENTION

For infection to occur, discharged seeds must become favorably placed at suitable sites on the host branches. The numbers of seeds that reach such sites and the manner in which they do so is a subject of some controversy.

MacDougal (1899) noted that discharged seeds of *Arceuthobium vaginatum* stick to branches or other objects they strike. Peirce (1905) observed that the majority of *A. campylopodum* seeds strike the needles of the host. He did not mention that seeds moved from needles but reported that penetration will not be successful unless the seed falls on a branch.

Dowding (1929) found that seeds of *Arceuthobium americanum* expelled at close range (6 feet or less) did not adhere to a cotton sheet or a spruce board. At greater distances, however, the seeds always adhered when they struck the target. She suggested that the seeds adhere to an object only when they land sideways, as they do when they begin to fall. Falling did not begin until seeds had traveled 6 feet or more.

Roth (1959) found that hard, smooth surfaces held close to mature plants were poor receptors when used for seed collection because the seeds glanced after striking. For this reason stems were poor receptors, whereas needles, which are resilient and offer a greater target area, accumulated seeds. Seeds remained on needles until the first rain, at which time their viscin layer swelled; they increased in mass and slid down needles to the base of the fascicle, or lodged at the open end of the fascicle sheath. If needles were inclined downward, the seeds slid from the tip and dropped off.

Methods

Studies on seed deposition and retention were conducted under natural field conditions at Pinecrest. Healthy, pole–size, dwarfmistletoe-free red firs and white firs were artificially inoculated by shaking clumps of female dwarfmistletoe shoots bearing mature fruit near a host tree so that the seeds were discharged into the crowns. Individual branches were tagged, examined, and the number and location of seeds were recorded, after which periodic examinations of retention and position of the seeds were made.

One tree of each species was inoculated in 1958. Each was open growing but adjacent to uninfected overstory and pole-sized trees. Three red firs and five white firs were inoculated in 1960. The red firs were located on an exposed lava cap and subject to strong winds, driving rain, and snow. The white firs were well protected by overstory and adjacent pole-sized trees.

The percent of total seeds disseminated that landed on hosts was not investigated in this study. Only seeds deposited on host trees were investigated.

Results

Results of the studies in 1958 and 1960 showed that most of the seeds that struck the host adhered to needles (fig. 6), with a few initially deposited on branches. Seeds remained on needles until the first rain, at which time the viscin layer swelled (fig. 7), then they became slippery and slid down the needles to the branches (fig. 8), or were washed off the host. Few seeds remained on needles after the first rain, whereas seeds initially deposited on branches generally remained in position.

Seed movement and position were observed in some detail on white firs inoculated in 1960. In this year the first substantial rain (0.52 inch) that followed inoculation and that was sufficient to cause seed movement occurred October 6–7. On October 7 observations showed that many seeds previously on needles had moved to the branch at the base of the needle on which they were formerly located. Some still remained on needles. A number were missing and undoubtedly had been washed off the host. For several hours after the rains, the seeds remained swollen and slippery.

On October 12, 5 days after the rain, with few exceptions the seeds were in the same positions as observed on October 7. They were no longer swollen and slippery but were dry and attached firmly to the host. From October 12 to May 1, 1961, more than 20 inches of rain and snow fell. However, few of the seeds that remained on the needles after the first rain in October had moved to branches by May. Thus, on October 6–7,

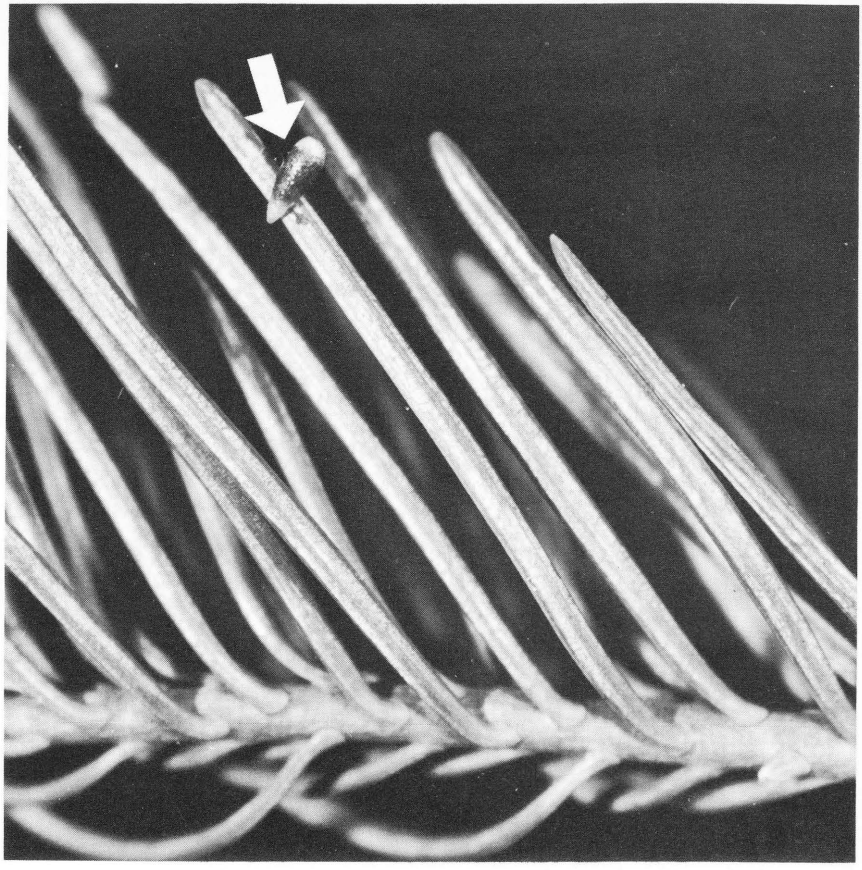

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Figure 6.—**Dwarfmistletoe seed on needle of red fir.**

in which about a half-inch of rain fell, most of the seed movement to branches occurred during a period of less than 48 hours.

The movement of seeds on needles was affected both by rainfall and the orientation of the needles. The needles of red fir are relatively short, stiff, and oriented predominantly upright. Most of the moist seeds slid down these needles to branches. Some of the needles of white firs are also oriented in a more or less upright position, but the somewhat longer needles of white fir frequently assume a horizontal to slightly drooping position. Seeds on needles in these positions generally either remained in place or were washed off.

In general, about half as many seeds moved along needles to branches of white fir as did to branches of red fir (fig. 9). For the dwarfmistletoe on white fir, this amounted to about 30 to 40 percent and for the one on red fir about 70 percent of the total seeds initially deposited. In 1958 on both hosts and in 1960 on white fir, nearly all seeds were still present on branches by the following summer. On the other hand, about half the seeds on branches of red fir in October 1960 were missing by May 1961. The reason for this high loss of seeds after placement on branches was not determined.

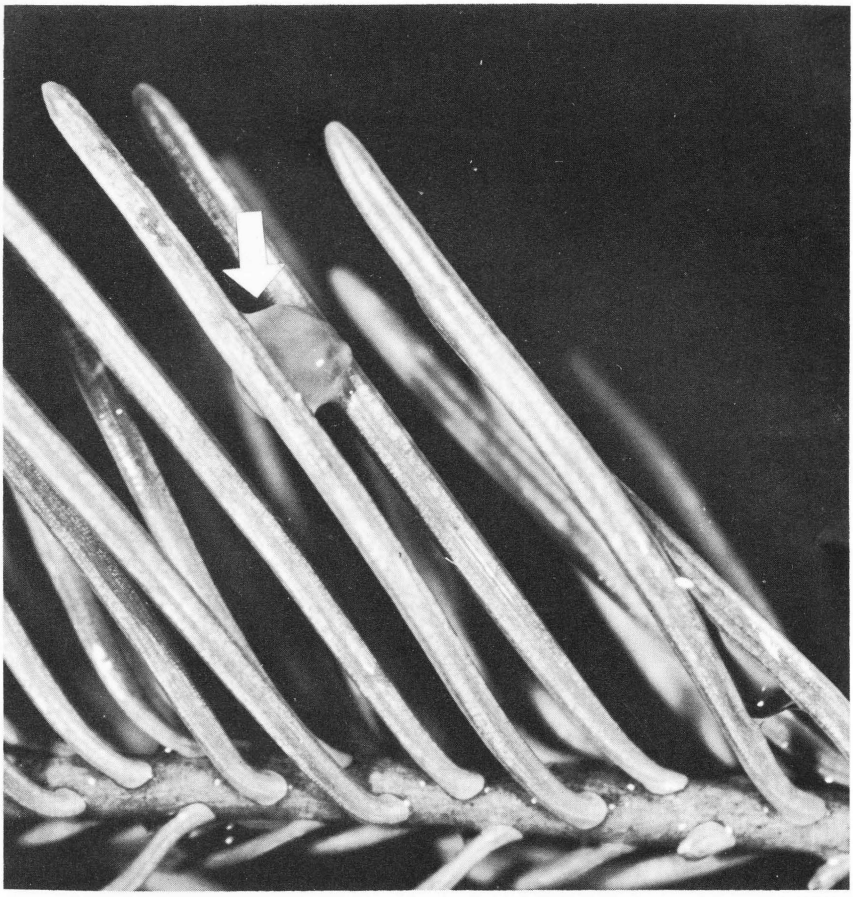

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Figure 7.—**Moist dwarfmistletoe seed on needle of red fir.**

Discussion

The present findings agree with those of Peirce (1905) and Roth (1959) in that the majority of the seeds of dwarfmistletoe initially were deposited on needles of the host. As to the fate of seeds on needles, however, results agreed more closely with those of Roth. Seeds on needles did not remain in place but during rains slid down needles and became favorably placed for infection. Roth found that 0.21 inch of rainfall was sufficient to cause seed movement and suggested that the approximate amount of rainfall necessary for movement is about 0.25 to 2.0 inches. In this study about 0.5 inch of rainfall was sufficient to cause movement and final placement of seeds.

As mentioned by Roth, the foliar and branching habits of a tree appear to influence seed movement and retention. Transfer of 70 percent of the seeds to branches of red firs and of 30 to 40 percent to white firs was considerably higher than the 20 percent Roth reported for ponderosa

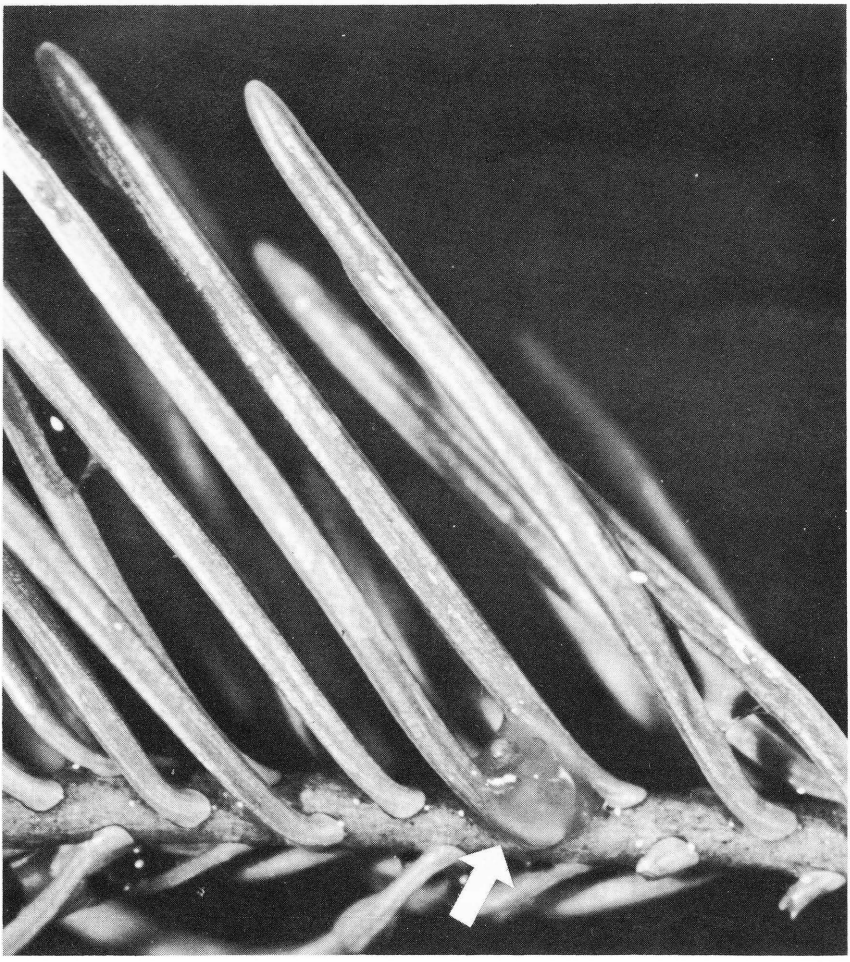

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Figure 8.—**Moist dwarfmistletoe seed on branch of red fir.**

pine. In firs the short, typically upturned needles are particularly suited for movement of seeds to branches. Attrition from strong winds or heavy snowpack, suggested by Roth as causing loss of seeds from ponderosa pine, may also have caused the high loss of seeds in 1960–61 from branches of the inoculated red firs growing on an exposed site.

SEED GERMINATION AND RADICLE GROWTH

Several general reports have been written on the germination of dwarfmistletoe seeds in nature. MacDougal (1899) noted in Arizona that ponderosa pines growing on the rims of canyons or on the tops of hills were most heavily infected with dwarfmistletoe and suggested that possibly greater relative humidity in these sites favored germination and establishment of the parasite.

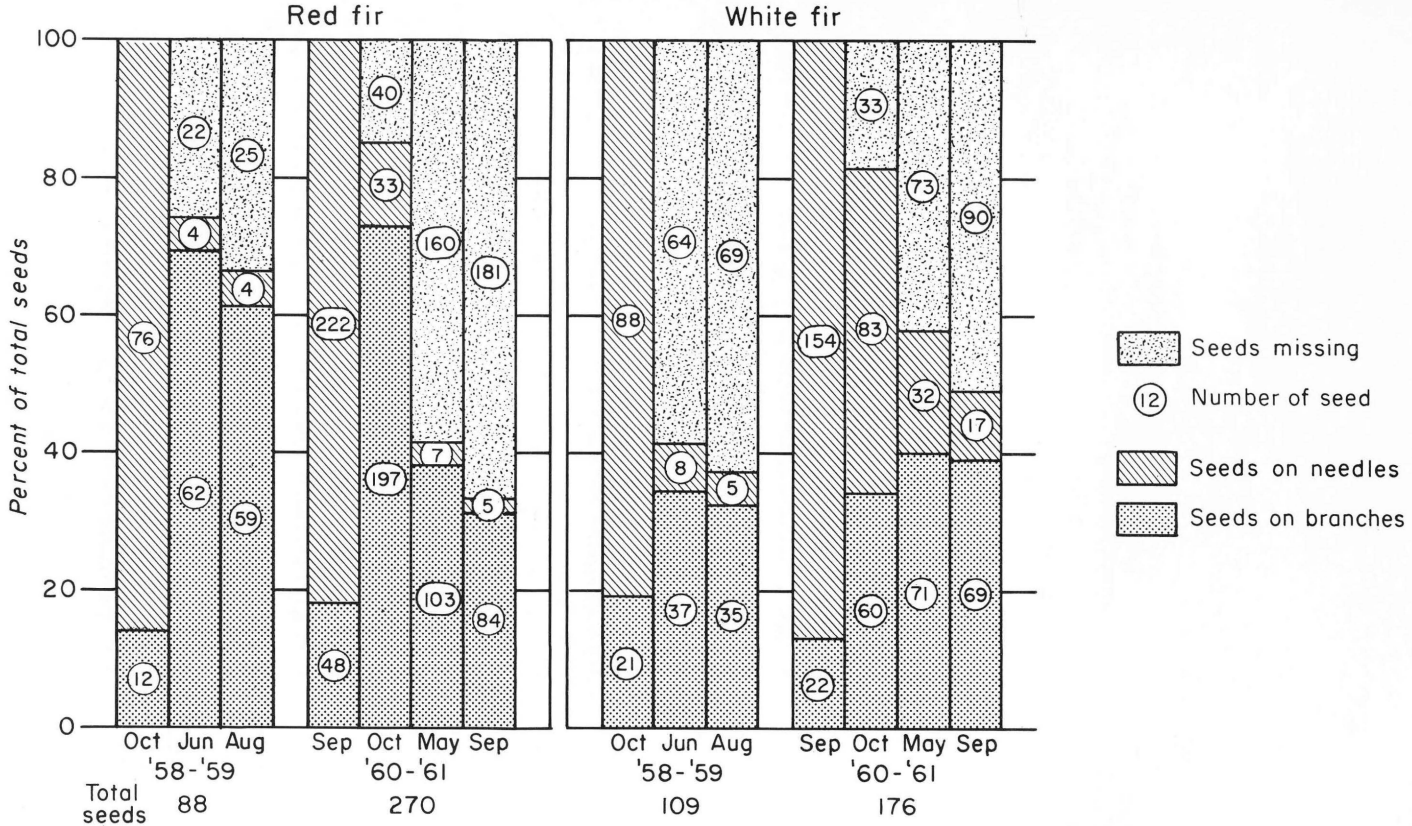

Figure 9.—**Retention and location of seeds of dwarfmistletoe on red fir and white fir (Pinecrest).**

Peirce (1905) was the first to observe and report on the germination of dwarfmistletoe seeds. Seeds of *Arceuthobium campylopodum* on Monterey pine in California germinated on any substrate, including living or dead needles of the host, dead branches, nonhost trees and shrubs, and fence boards. He suggested that damp, moderately cool air was necessary for germination. Heinricher (1915b) observed germination of *A. oxycedri* in the field chiefly in March. Kuijt (1955) stated that germination of the North American species occurs in the fall or early spring; in fall germination, growth of the radicle was often arrested during the winter but resumed again with more favorable conditions.

Weir (1918), reporting results of inoculations with some of the North American species of dwarfmistletoe on various hosts, stated that the seeds required a period of rest before germination and that low winter temperatures seemed beneficial. On the other hand *Arceuthobium vaginatum* apparently germinates within a few weeks after dissemination, provided environmental conditions are favorable (Hawksworth 1961). According to Hawksworth (1961), germination takes place in August and September during the summer rains. Forty to 50 percent germination was noted for naturally deposited seeds.

Growth of the dwarfmistletoe radicle, although a distinct stage of development of the parasite, may be discussed with germination. For the most part, little is known about radicle growth. Most investigators have found that radicles grow along the surface of a branch until an obstacle is met before a structure ("holdfast") is formed (Gill 1935, Peirce 1905, Hawksworth 1961, Kuijt 1960). Holdfast formation presumably is essential for penetration, but Heinricher (1915b) reported that radicles of *Arceuthobium oxycedri* may or may not grow in length and that holdfast development is not always necessary for penetration.

Thus, the role of holdfast formation in penetration has not been clearly demonstrated. Studies on holdfast formation and its role in penetration and infection will be discussed in the following section.

Methods

Observations on the germination of dwarfmistletoe seeds on red fir and white fir were made in the field. Trees at Pinecrest were inoculated in 1958 and 1960. Seeds placed on branches of red firs and white firs at Latour State Forest in 1958 were also observed for germination.

Results

After dissemination, the seeds remained on hosts from 4 to 5 months before they began to germinate. At the beginning stage of germination, germinated seeds were difficult to distinguish from ungerminated seeds. Examination showed that the hairlike fibrillous strands of the viscin layer of the seeds formed a protective coating over the radicular end. Young growing radicles were frequently hidden from view beneath this coating, and it was necessary to remove seeds from branches to determine if germination had occurred. Examination of 65 untagged seeds removed from white fir branches at Pinecrest in the middle of February 1959 showed that 39 percent were in the early stage of germination. Later in the year, however, growing radicles were readily observed without removing the seed from the host (fig. 10). Seeds on needles germinated as readily as did seeds on branches.

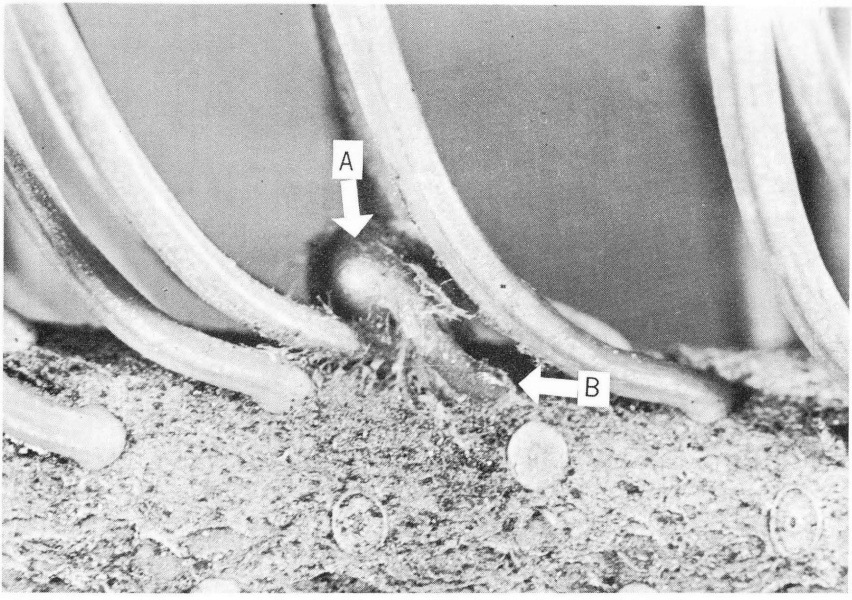

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Figure 10.—**Germinated seed of dwarfmistletoe on red fir: *A*, Seed; *B*, radicle.**

By June, about 9 months after seed dispersal, more than 70 percent of the seeds on white fir, and more than 80 percent on red fir, had germinated. Only an additional 4 percent of the seeds on white fir and 3 percent on red fir germinated between June and August. No germination occurred after August.

A year after discharge 70 to 90 percent of the seeds on hosts at Pinecrest and Latour had germinated (table 1). The percent was slightly higher on both fir species in 1958 than in 1960. No striking differences were observed in percentage germination between the red fir and white fir dwarfmistletoes.

The growth of radicles after germination was also observed in the field. The extent of linear growth of radicles depended mainly on the location of the seeds on the hosts and whether an obstacle was encountered. Radicles on the smooth surface of needles failed to form holdfasts and continued linear growth until they exhausted their nutrient supply. On branches the extent of linear growth varied considerably. Seeds located with their radicular ends pressed to a needle base usually produced short radicles and formed holdfasts shortly after germination. Radicles of seeds not at a needle base usually grew for varying distances before meeting a needle base or bark irregularity. Thus, the length of a radicle and duration of the period of linear growth before holdfast formation depended primarily on the location of the seed on the host and the time required for the radicle to encounter an obstacle.

Holdfasts always developed at the apical part of the radicle and generally began as small knoblike swellings, often irregularly shaped (fig. 11). For example, at the base of a needle a holdfast usually developed into a wedgelike structure that conformed to the angle formed by

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TABLE 1.—*Germination of dwarfmistletoe seeds by autumn of the year following dispersal*

Inoculation date and host	Seeds remaining	Seeds germinated
	Number	*Percent*
Red fir:		
Pinecrest:		
1958	63	87
1960	89	69
Latour:		
1958	75	88
White fir:		
Pinecrest:		
1958	40	80
1960	86	76
Latour:		
1958	20	90

the needle and branch. On the other hand, holdfasts that formed on the surface of branches at bark cracks or branch forks were more or less dome shaped with the lower surface usually flat and appressed to the bark surface.

Of the 181 radicles that produced holdfasts in 1958 and 1960, 169—or 93 percent—formed at the base of needles. The remaining 7 percent occurred at branch forks or on the surface of the branches at bark cracks.

Radicles began holdfast development from about April to July, depending on the time of germination and the distance that a given radicle grew before encountering an obstruction.

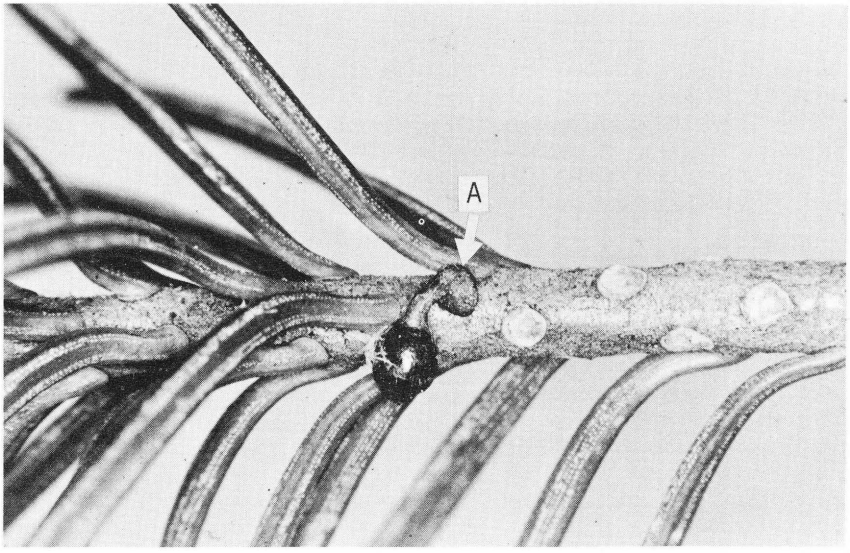

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Figure 11.—**Holdfast (*A*) formed on branch of red fir at the base of a needle.**

The number of radicles of the red fir dwarfmistletoe that produced developing holdfasts, at the end of specified months in 1958, was as follows:

Holdfast:	*February*	*April*	*June*	*August*
Developing	0	4	48	45
Not developing	25	75	7	9

Holdfasts generally persisted for a considerable time after development. The majority of them were present and apparently still living until winter of the same year. By summer of the following year, however, most holdfasts were dried and dead. Holdfast formation will be discussed further in the following section on penetration and infection.

Discussion

Unlike the germination of *Arceuthobium vaginatum*, which occurs within a few weeks after seed discharge, germination of *A. campylopodum* on fir did not take place until late winter (about 4 months after discharge). The characteristically dry fall weather in California appears to inhibit germination until winter rain and snow provide adequate and sustained moisture. However, under cold winter conditions even in the presence of adequate moisture, germination proceeds slowly and is not apparent until early spring.

The report by Weir (1918) that seeds require a rest period before germination was not confirmed for dwarfmistletoe on firs. Also, no evidence suggested that seeds germinate in the fall, overwinter, and resume growth in spring as reported by Kuijt (1955). The period through which seeds germinated, as indicated by the appearance of radicles, was much greater than the 1- to 2-month period reported for *Arceuthobium vaginatum* (Hawksworth 1961). Germination was observed as early as February and as late as June. Percent germination of *A. campylopodum* on fir was also consistently higher than that reported for *A. vaginatum* (Hawksworth 1961). Whether this reflects differences in viability of seeds, duration of suitable conditions for germination, or variations in prevalence of mold fungi, seed-eating insects, and other damaging agents is not known.

Observations on fir dwarfmistletoes support previous reports that radicles must encounter an obstacle, usually a needle base, before a holdfast develops. On firs no evidence suggested that radicles may grow on the surface of stems for as long as a year before penetration as reported for *Arceuthobium vaginatum* on pine by Gill and Hawksworth (1954).

PENETRATION AND INFECTION

The first detailed studies of holdfast formation and penetration by a mistletoe were made by Thoday (1951) on *Viscum album* L. He described the following stages of holdfast formation and penetration:

(1) A holdfast becomes attached by means of marginal "papillae," which adhere to the host surface; (2) growth of the walls of the holdfast, growth of new papillae, and the collapse of a subcortical zone of cells in the holdfast, which may have a contractile effect, result in opening a slit through which the "primary haustorium" penetrates; (3) the penetrating haustorium develops into a narrow, oval wedge, its forward penetrating edge remaining meristematic, sometimes with a fringe of papillae.

Kuijt (1960), who investigated holdfast formation in *Arceuthobium*, noted that when the elongating radicle encounters an obstruction a fundamental change develops in its growth pattern. The meristematic activity of the apex spreads to a zone somewhat subterminal. The holdfast is formed by the combined activity of the subterminal "growth centers" oriented in lateral direction. However, the integrity of the superficial layer is maintained even in well-developed holdfasts. Where a holdfast does not become securely attached to the host, a small but regular apical meristem may be "reconstituted." According to Kuijt, linear growth of a radicle can resume from a reconstituted apical meristem.

Peirce (1905) and Cohen (1963) have considered the penetrating structure of the parasite to be a true but highly specialized primary root. Cohen reported the following sequence of events to take place during penetration: Upon encountering an obstruction the root apex is deflected downward. Surface initials grow between bark cracks and attach the appressed root to the branch. The root apex continues its forward extension until it is impeded by the bark. Addition and growth of derivatives of the central initials cause the root apex to bulge, and a holdfast is formed. Further growth of the central initials causes "tension" to build up, and obliteration of cells toward the periphery of the holdfast occurs. As penetration continues, the subapical zone of initials is left behind. Thereafter, the penetrating structure consists only of surface and procambial initials. Only the procambial initials penetrate the host and establish infection.

The mechanism of penetration by dwarfmistletoes has been the subject of some controversy. Most investigators who have studied penetration in other genera of mistletoes have suggested that both mechanical and chemical action take place (Cannon 1904, McLucky 1923, Menzies 1954, York 1909, Thoday 1951). Mechanical and chemical penetration of hosts by dwarfmistletoes has also been suggested (Peirce 1905 and Kuijt 1960). However, Weir (1916), stated that mechanical force is the primary mechanism of penetration by the parasite.

Practically no information is available on the time of the year when holdfast formation, penetration, and infection occur. A report by Dowding (1929) suggested that penetration by *Arceuthobium americanum* does not occur until late in June the year following seed dispersal.

Infection and initial symptom expression by dwarfmistletoe have also been inadequately investigated. Most investigators consider the presence of shoots of the parasite to be the first indication of infection (Peirce 1905, Gill and Hawksworth 1954, Weir 1918). However, Heinricher (1915b) first noticed a chlorotic appearance of buds of juniper (*Juniperus communis* L.) immediately adjacent to penetrating seedlings of *Arceuthobium oxycedri*. Hawksworth (1961) and Wagener (1962) observed that swelling at the point of infection usually preceded shoot production by the parasite.

Reports indicate that the proportion of seeds of dwarfmistletoes that reach the infection court and cause infection is rather small. Heinricher's (1915b) study on *Arceuthobium oxycedri* showed that relatively few seeds of the total number placed on juniper branches caused infection. The greatest number of infections obtained on a single host was 38 out of 300 seeds, or slightly more than 10 percent. Of 1,500 seeds planted on 5 hosts, about 8 percent produced infection. Somewhat similar results were obtained by Weir (1918) from artificial inoculations of various conifers with seeds of several species of *Arceuthobium* in North America. In none of Weir's tests, conducted in the field and under greenhouse conditions, was more than 10 percent infection obtained. Results of inoculations of

ponderosa pine with seeds of *A. vaginatum* over 3 successive years showed that of all seeds deposited on susceptible tissue only about 5 percent became established (Hawksworth 1961).

Relatively little is known about the factors that influence infection, but environmental conditions have been reported to be important. Wagener (1961) suggested from field observations that partial sunlight is more favorable for establishment of *Arceuthobium campylopodum* on ponderosa pine and Jeffrey pine than relatively full or continuous sunlight, but he did not mention the stage in the infection cycle of the parasite when high light intensities inhibited establishment.

The resistance of older branches to infection has been a subject of some investigation. Weir (1916, 1918) reported that the basis of resistance lies in the failure of the parasite to penetrate the bark of older branches. For instance, Weir (1916) found no evidence that dwarfmistletoe could penetrate the smooth bark of Douglas-fir or older branches of ponderosa pine and western larch (*Larix occidentalis* Nutt.). He removed radicles and holdfasts and noticed that only a barely perceptible indentation was sometimes present. When he inoculated segments of ponderosa pine branches 1 to 10 years old, infection occurred only on segments 1 to 3 years old. Weir (1918) demonstrated experimentally, however, that by scraping away the "cork" on branches as old as 7 years it was possible to secure infection. On the other hand, Hawksworth (1961) reported that branch segments of ponderosa pine 1 to 9 years old were naturally infected by *Arceuthobium vaginatum* but that the younger segments were more susceptible. Observations of *A. americanum* on lodgepole pine by Hawksworth (1954) showed that the parasite is capable of penetrating and infecting branches more than 60 years old.

Methods

To study holdfast formation and penetration of firs by dwarfmistletoe, small parts of host branches bearing germinated seeds were collected at monthly intervals at Pinecrest from June through October 1958. The samples were fixed in formalin-acetic acid-alcohol (FAA), imbedded in paraffin, and transverse and radial sections were made on a rotary microtome at 12–15 microns. The sections of each sample were then affixed in serial arrangement to microscope slides with Haupt's adhesive, stained with safranin-fast green, and mounted in "Permount."

Additional sections, cut as described above, were used for histochemical tests to determine if pectic substances or suberized tissues of the host were destroyed or removed during penetration. To test for the presence of pectic materials, sections were treated with a 1:5000 aqueous solution of ruthenium red for about 5 minutes, washed in water, and mounted in glycerin. To detect suberized tissue of the host, sections were treated with a saturated solution of Sudan IV for 30 minutes, washed in water, and mounted in glycerin. All sections were examined with a compound microscope.

At Pinecrest red firs bearing germinated seeds were used to determine the time of the year when infection occurs. About 75 to 150 seeds with developing holdfasts were cut from branches with a sharp razor blade each month from June to October 1958. Care was taken to remove all parts of seeds and holdfasts from the surface of the branches. The points on the branches where seeds had been removed were marked and observed for infection. Seeds left in position on branches as controls were also marked and observed for infection.

Observations on percentage infection and symptom development were made periodically for 5 years on firs at Pinecrest and at Latour inoculated in 1958.

Additional firs bearing naturally deposited seeds were observed in the field and material was collected for microscopic examination to investigate factors involved in infection.

Results

Penetration

Holdfast formation began as a result of periclinal divisions of cells of the protoderm in the part of the radicle immediately behind the apical meristem (fig. 12,*A*). These newly initiated parenchymatous cells repeatedly divided in all planes and enlarged. As division occurred and cells enlarged, the cell walls thickened slightly and intercellular spaces developed.

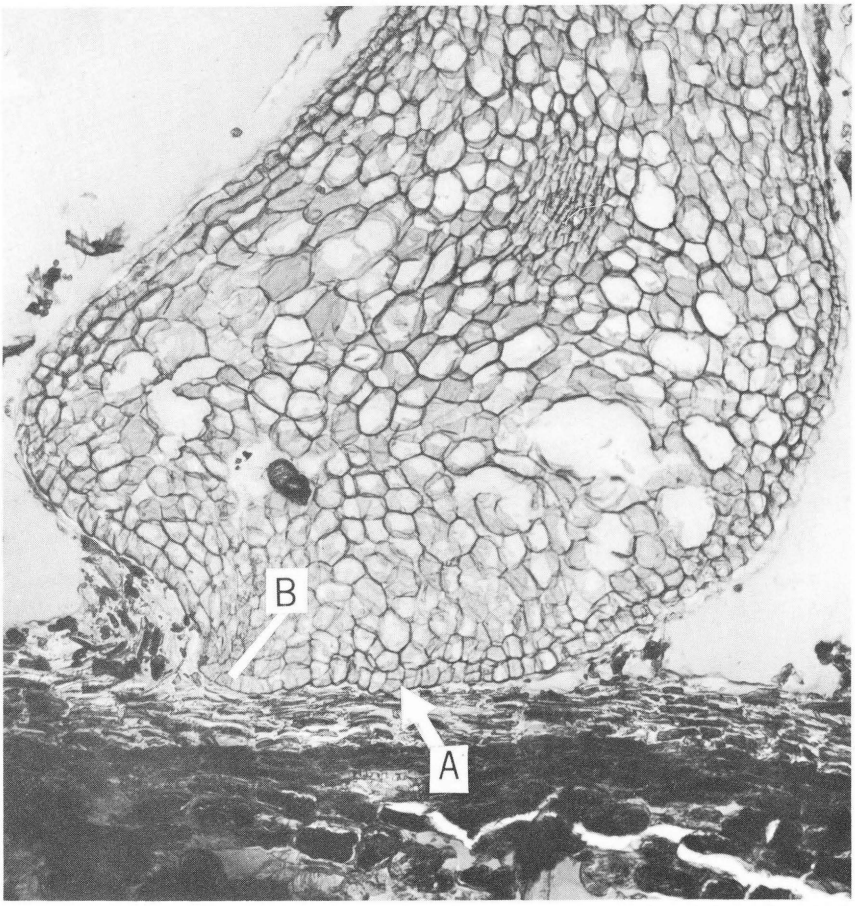

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Figure 12.—**Median section of a young holdfast of dwarfmistletoe on red fir: *A*, Periclinal divisions of the protoderm in the subapical lateral part of the radicle; *B*, protuberance that develops into a penetrating structure. (× 105)**

Both the increase in cell size and number contributed to the formation of the enlarging knoblike holdfast. Anticlinal divisions of cells of the protoderm occurred as the holdfast enlarged and the protoderm maintained its single cell layer organization.

During formation of the holdfast, the apical meristem of the radicle retained its meristematic appearance. Although relatively few divisions were observed, cells of this region had dense cytoplasm, were thin walled, and contained enlarged nuclei. Intercellular spaces were absent.

A strand of primary xylem was observed in developing holdfasts, and appeared to be continuous with the vascular strand in the radicle.

As the holdfast enlarged and became more closely appressed to the surface of the host branch, cells at the margin divided, became elongated, and penetrated the bark (fig. 13,*A*). These somewhat elongated cells did not penetrate into the living tissue of the host, but presumably functioned to secure the holdfast to the branch.

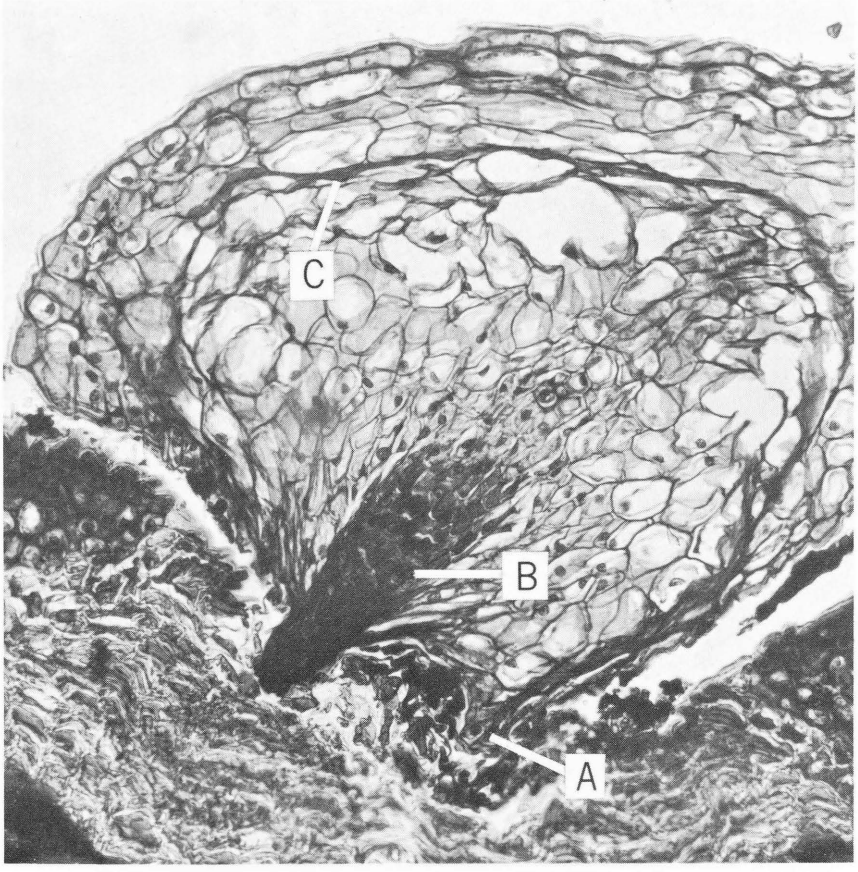

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Figure 13.—**Median section of a well-developed holdfast of dwarfmistletoe on red fir: A, The cells at the margin of the holdfast that aid in securing the structure to the branch; B, the darkly stained, highly meristematic penetrating structure: C, the layer of crushed cells within the holdfast. (× 107)**

The apical meristem, which remained active during holdfast development, initiated the structure that penetrated the host. This structure, referred to here as a "penetrating structure," was first recognized as a slight protuberance on the appressed surface of the developing holdfast (fig. 12,*B*). Only one penetrating structure developed from each holdfast. Subsequent enlargement of the structure took place by the repeated division and enlargement of cells of the meristem. Anticlinal divisions of the cells in the epidermal layer of the structure during early development maintained its integrity.

Repeated divisions of these apical cells and division and elongation of cells behind the apex caused the structure to become tightly pressed against the bark. As it enlarged and began to penetrate the bark of the host, the structure developed into a broadly wedge-shaped structure (fig. 14). Observed in another plane of view, however, it sometimes appeared narrowly wedge-shaped (fig. 13,*B*). Penetration appeared to be effected by the wedging action of the enlarging structure. The force that the parasite exerted caused crushing of the bark cells (fig. 14,*A*). Also, during penetration, cells at the surface of the meristem were crushed (fig. 13,*B*). Repeated division and enlargement of cells behind the apex of the penetrating structure increased the width of the structure and resulted in additional crushing of the bark. During initial penetration no cell differentiation in the structure was observed. The bulk of the cells were thin walled and parenchymatous. Cell differentiation occurred after initial penetration.

Procambium, primary xylem, and ground meristem were present in well-developed penetrating structures (figs. 14 and 15). Primary xylem usually was restricted to the basal part and procambium observed only in the central region of the structures, suggesting an acropetal sequence of differentiation.

During penetration of the bark by the parasite, a zone of crushed cells appeared in the holdfast (figs. 13,*C*, 14,*E*). This zone of crushed cells was regularly observed in holdfasts and appeared to be the result of strong mechanical forces placed upon the cells within the holdfast by the penetrating structure as it forced its way through the suberized tissues of the host.

The penetrating structure retained its apical meristem and wedgelike appearance during the entire course of penetration through the outer bark. However, upon reaching the living tissues of the host, the structure lost the apical pattern of cell division and its wedgelike appearance and usually developed into an irregularly shaped mass of tissue (fig. 15). This mass of tissue appeared to develop as a result of repeated division and enlargement of cells at the periphery, as well as at the advancing margin of the structure. No distinct periderm was observed on the developing structure, but rather the advancing margin appeared to consist of elongated fingerlike cells.

Extension of the parasite within the cortex appeared to be effected by the intrusion of these fingerlike cells between the cells of the cortex (figs. 14,*F*, 15,*C*, 16,*A*). As they divided and enlarged, adjacent host cells were crushed (fig. 16,*B*). Considerable displacement of the host tissue by parasite cells was also observed; however, killing of host cells in advance of the parasite was not detected. In none of the specimens examined had differentiation of the cortical strands of the endophytic system occurred. Thus development of the parasite subsequent to the stage shown in figure 15 was not investigated in this study.

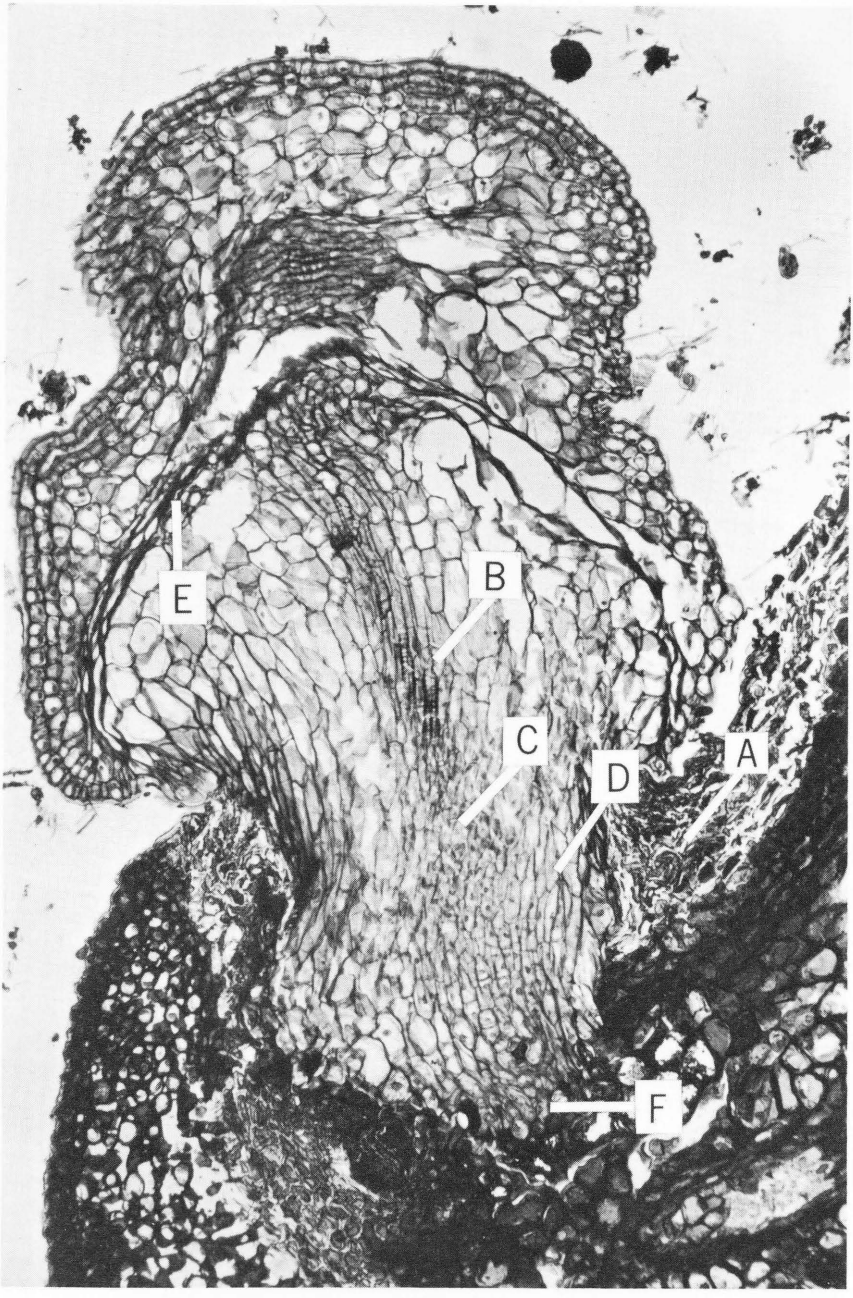

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Figure 14.—Active stage of penetration of red fir by dwarfmistletoe: *A*, Crushed cells of the bark; *B*, strand of primary xylem; *C*, procambium; *D*, ground meristem; *E*, layer of crushed cells in the holdfast; *F*, fingerlike cells at the advancing margin of the penetrating structure. (× 86)

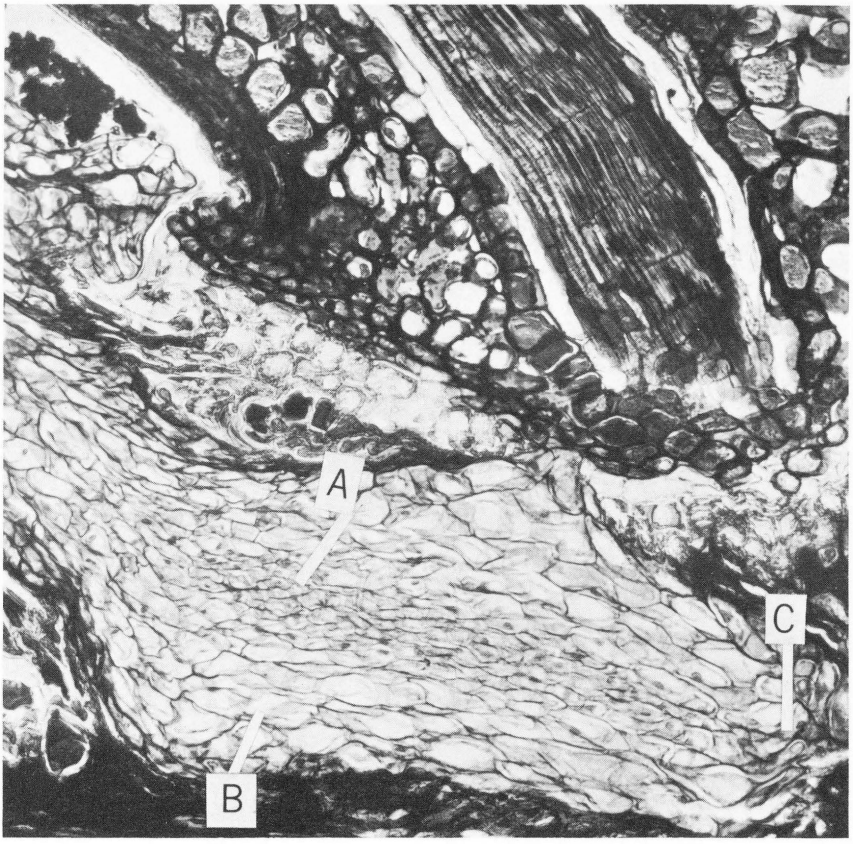

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Figure 15.—**Advanced stage of penetration of red fir by dwarfmistletoe: *A*, Procambium; *B*, ground meristem; *C*, fingerlike cells at the advancing margin of the penetrating structure. (× 155)**

Microscopic examination of dwarfmistletoe at various stages of penetration indicated that the process of penetration was mechanical. No enzymatic digestion of host tissues was detected. Bark penetrated by the parasite, stained with Sudan IV and examined microscopically, showed no evidence of breakdown or loss of suberized materials. Crushed cells were observed, however. No loss of pectic materials from within cells or from the middle lamella of host tissues penetrated by the parasite was detected with ruthenium red.

Microscopic examination of sections of host branches bearing germinated seeds in June and July of the year following seed discharge showed that 75 percent of the seeds had formed holdfasts and had begun penetration (fig. 17). All but one of the seeds with holdfasts observed in these months were penetrating only outer bark; the one exception had penetrated beyond the outer bark and had reached the living tissue of the host. In August and September 80 percent or more of the seeds were penetrating branches, and about half had reached the living tissues. All seeds observed on branches in October had formed holdfasts and were penetrating. Sixty percent of these had penetrated to living tissue.

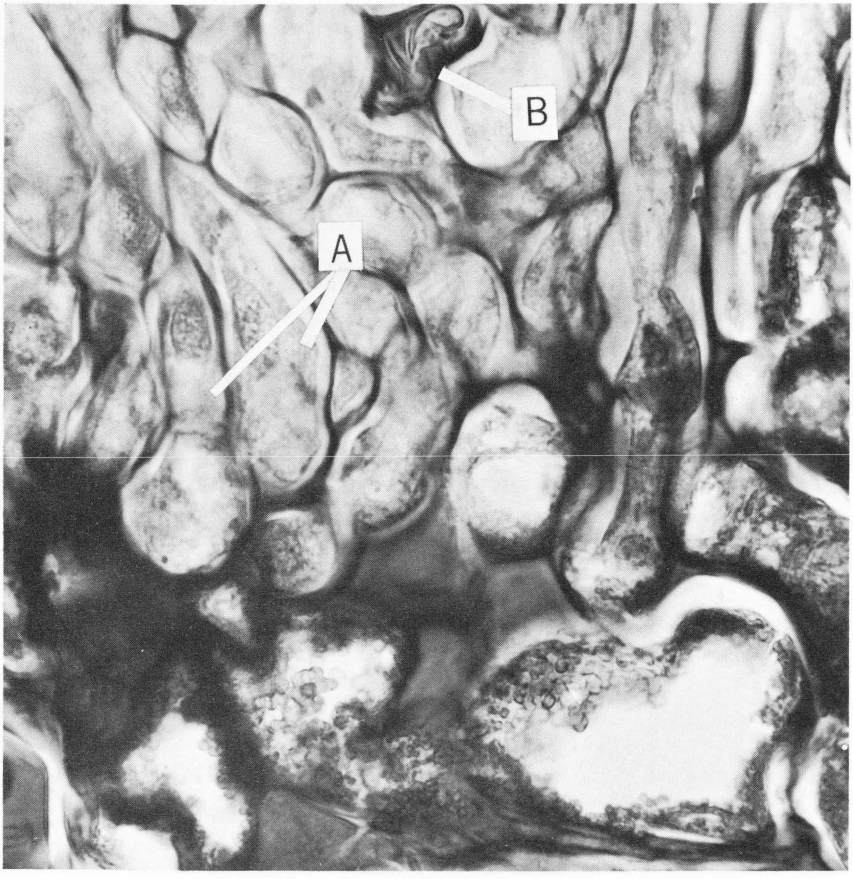

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Figure 16.—**Advancing margin of the penetrating structure shown in fig. 14: *A*, Elongate, fingerlike cells of the parasite, within the host cortex; *B*, cortical cell being crushed by the parasite. (× 600)**

Infection

The first noticeable symptom of infection of firs was a localized swelling of the branch at the site of penetration (fig. 18). Details of symptom development will be discussed in the following section on incubation period. The exact site of penetration was readily recognized in many cases because the dried seeds and radicles were present at the time swelling occurred. The seeds, when present, were located near the center of the swollen part of the branch (fig. 18).

Occasionally small dwarfmistletoe shoot buds appeared from within the bark of a branch at about the same time swelling occurred (fig. 19). Shoot buds were not observed before swelling, however. Frequently buds appeared a year or more after initial swelling.

At Pinecrest a very low percent of the seeds initially deposited on red firs and white firs caused infection (table 2). Even for the seeds which

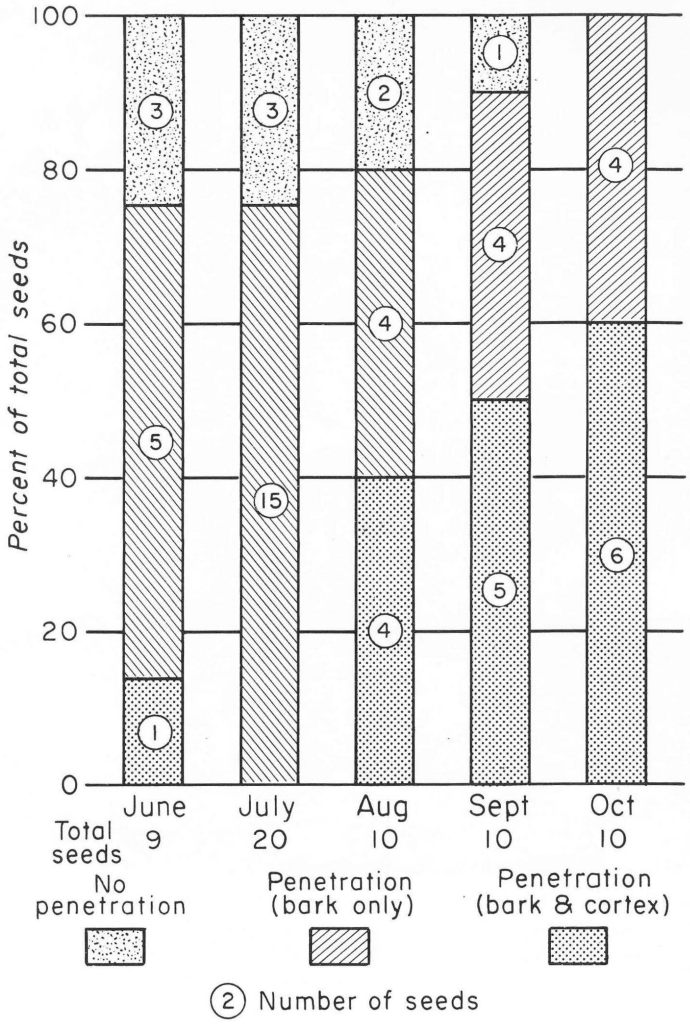

Figure 17.—**Penetration of red fir by dwarfmistletoe at Pinecrest, monthly intervals, June through October 1959.**

formed holdfasts and penetrated, percent infection was low (less than 6 percent).

At Latour no infections resulted from seeds placed on white firs. The number of seeds that survived and formed holdfasts on this host species was low, but on red firs, percent of infection was high: 34 percent of the seeds initially placed on trees and 45 percent of the seeds that formed holdfasts caused infection.

Time of infection is shown in table 3. No infection resulted from seeds removed in June and July. Of the seeds removed in August, however, 5 percent resulted in infection. A slightly lower percent was noted for

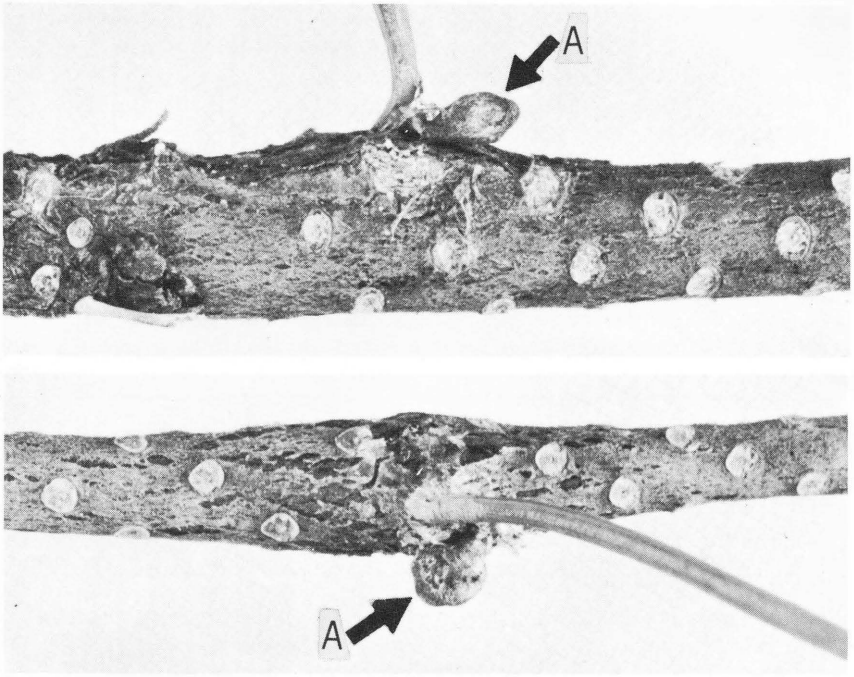

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Figure 18.—Initial swelling of red fir by dwarfmistletoe; persistent needle and dried seed (*A*) of the parasite indicate site of penetration. (× 3)

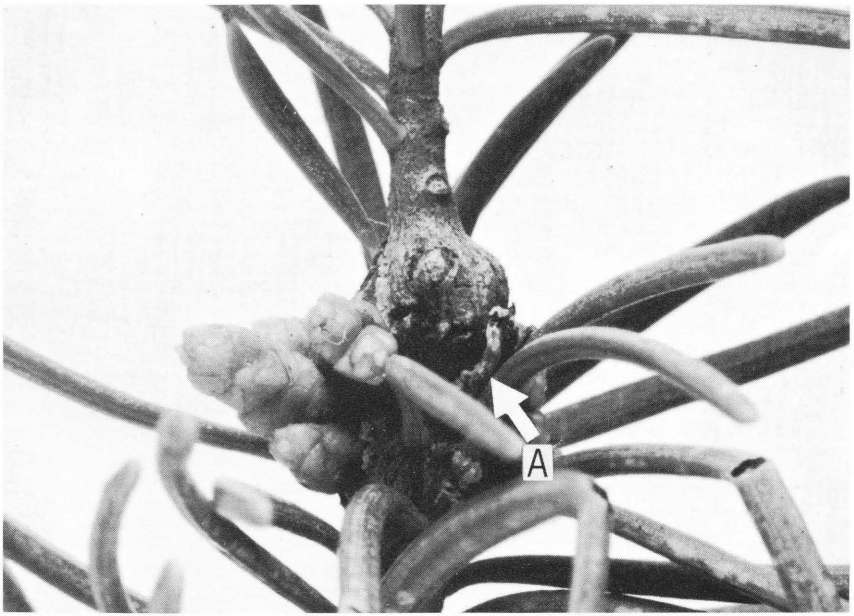

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Figure 19.—Young dwarfmistletoe plant growing on red fir. First shoots arise from the area of swollen branch tissue. The dried radicle of the seed (*A*) is still present on the branch. (× 3)

seeds removed in September. The seeds left in place as controls showed only a slight increase in percentage infection over that of the seeds removed in August and September.

TABLE 2.—*Dwarfmistletoe infections appearing by 1963 on firs inoculated in 1958*

Host	Total seeds	Seeds with holdfasts	Infections		
			Number	Percent of total	Percent with holdfasts
Pinecrest:	*Number*	*Number*			
Red fir	88	51	3	3.4	5.9
White fir	109	27	1	.9	3.7
Latour:					
Red fir	100	75	34	34.0	45.3
White fir	100	18	0	0	0

TABLE 3.—*Infection of red fir by dwarfmistletoe: Seeds removed at monthly intervals, June through September 1959 (Pinecrest)*

Month	Seeds removed	Infections	
	Number	*Number*	*Percent*
June	75	0	0
July	148	0	0
August	129	7	5
September	104	3	3
Control [1]		8	8

[1] 106 seeds, not removed.

Field observations showed that on red firs and to a lesser degree on white firs, seeds penetrating branches at the base of a needle frequently killed the needle. These observations suggested that the death of needles during penetration might influence infection. To investigate the effect of needle dying on infection, branches bearing seeds penetrating at the base of living needles and also branches bearing seeds at the base of dying needles were tagged and observed for infection for about 4 years. All tests were established on naturally infected red firs growing at Pinecrest. The results were as follows:

Location of seed	*Seeds observed*	*Infections*	*Infection*
Base of:	*Number*	*Number*	*Percent*
Living needle	46	8	17
Dying needle	94	1	1

These results showed that a considerably lower percentage of infection occurred from mistletoes penetrating at the base of dying needles than from those penetrating at the base of living needles.

A subsequent study was made to ascertain why needles died during penetration and to investigate the factors of the host-parasite relationship that influenced infection. Branch segments bearing penetrating mistletoes at the bases of healthy and dying needles were sectioned, stained with safranin and fast-green as previously described, and observed for penetration and infection.

This investigation showed that death of a needle was usually associated with needle abscission, apparently caused in part by mechanical forces exerted on the needle and branch during holdfast formation and penetration. Abscission often occurred when a holdfast became securely attached to the branch at the base of a needle and particularly when a holdfast became tightly wedged in the acute angle formed by a needle and branch. In addition to the mechanical forces placed upon a needle, the wedgelike penetrating structure also ruptured host tissue at the base of a needle thus weakening the attachment of the needle. Apparently both the mechanical force exerted upon the needle by the developing holdfast and the rupturing of the branch tissue by the penetrating wedge were involved in causing abscission. Death ultimately resulted from the breaking of the vascular connection of the needle with the branch.

The host reacted to these stresses placed upon the needle and branch tissue by producing wound cork and by forming an abscission zone (fig. 20). The abscission zone, composed of a rather thick layer of suberized tissue, appeared in most instances to inhibit penetration of the parasite into the living tissue of the branch.

Occasionally a needle would be killed without being mechanically broken loose from the branch. In these instances microscopic examinations showed that little wound cork had formed and that the parasite had reached the living tissues of the host. Killing of the needle evidently was caused by the crushing of the leaf trace by the enlarging rootlike system of the parasite within the cortex (fig. 21). Death of these needles apparently had no effect on infection, since the parasite was already well established within the living host tissues.

The effect of branch age on infection by dwarfmistletoe was also tested. At Latour in 1958 the seeds used to inoculate red firs were placed at the base of needles on branch segments 1 to 5 years old. The percent infection that occurred on each age of segment as determined by 5 years' observations was as follows:

Branch age	*Seeds with holdfasts*	*Infections*	*Percent infection*
Years:	*Number*	*Number*	
1	18	6	33
2	15	8	53
3	17	9	53
4	15	6	40
5	10	5	50
Total, all ages	75	34	45

Results of the test show no marked difference in susceptibility to infection among branch segments of red fir from 1 to 5 years of age.

F-513370

Figure 20.—Section of part of a branch of red fir in which penetration by dwarfmistletoe was unsuccessful: *A*, The penetrating structure of the parasite that caused abscission of the needle; *B*, abscission zone; *C*, wound cork; *D*, leaf trace. (× 108)

F-513371

Figure 21.—Section of branch of red fir penetrated by dwarfmistletoe: *A*, Leaf trace being crushed by *B*, the advancing margin of the penetrating structure. (× 600)

Discussion

The general morphological features of a developing holdfast of *Arceuthobium* were found to be similar to those reported by Thoday (1951) for *Viscum*, and by Kuijt (1960) and Cohen (1963) for *Arceuthobium*. Disagreement exists, however, in the manner in which penetration occurs. The concept of Thoday that "marginal papillae" produced by the holdfast progressively lift and disintegrate the periderm until a slit is formed through which penetration occurs was not observed. Rather, penetration of firs by *Arceuthobium* appeared to be more nearly in agreement with that reported by Cohen. He stated that only the procambial initials of the parasite invade the host tissue, all other initials presumably being crushed or left behind during penetration.

Results of the present study, however, indicated that the penetrating structure consists of at least two different primary meristematic tissues, a procambium and a ground meristem. During early development, the cells of the structure are prosenchymatous, the long axis oriented in the direction of penetration. At this stage all cells of the structure may appear to be procambial initials. Penetrating structures in more advanced development, however, had a ground meristem as well as a procambium and primary xylem. Therefore, to classify the primary meristematic tissues only on the basis of morphology during early penetration may be an error.

An obliterated zone of cells in the holdfast as recorded by Thoday (1951) and Cohen (1963) was also observed in *Arceuthobium* in the present study. Thoday suggested that the zone of collapse, subcortical cells is the result of contractile action. Cohen reported that the crushed cells in the periphery of the holdfast are the result of tension built up within the holdfast. Present observations indicate that contractile action did not occur and that compression rather than tension obliterated the inner cells of the holdfast as the penetrating structure forced its way into the host.

The studies reported here agree with those of Weir (1916) in that penetration of the host by dwarfmistletoe is primarily a mechanical process. No evidence was obtained to suggest obvious chemical breakdown of tissues or separation of cells during penetration. However, until detailed studies using more precise methods of detecting chemical or enzymatic action are employed, the role of such substances in the process of penetration can not be ruled out.

Penetration of firs by dwarfmistletoe occurred over a period of several months. Undoubtedly the rather long period during which seeds formed holdfasts was partially responsible for the extended period of penetration. Even after holdfast formation, the length of time for penetration by the parasite varied from about 2 to 4 months and occurred in summer and fall. Possibly such factors as vigor of the seed, angle of penetration, and thickness of host bark have a marked influence on the length of time necessary for the parasite to penetrate to the living tissues of the host.

Initial symptoms and signs of infection of firs by *Arceuthobium campylopodum* were similar to those reported by Hawksworth (1961) and Wagener (1962). Localized swelling of a branch at the site of penetration was the first symptom and was nearly always quite conspicuous before shoots developed. Shoots of the parasite were not observed in the absence of branch swelling as had been reported by Peirce (1905)

and Weir (1918). Also, no chlorosis of buds or foliage near the site of infection was observed as had been reported by Heinricher (1915b) for juniper infected with *A. oxycedri.*

In all but one instance only a small percent of the total number of seeds deposited on firs caused infection. The generally low level of infection agrees with that reported by other investigators (Heinricher 1915b, Weir 1918, Hawksworth 1961). The exception in which percent of infection was high occurred in tests on red firs at Latour in 1958. Interestingly, no infections resulted from inoculations of white fir in the same area and in the same year. The reasons for the differences in percent infection of firs at the two study areas and between fir species in a given area are unknown. It seems unlikely that climatic factors alone are responsible for the differences, particularly at Latour. Natural differences in susceptibility to infection among trees or groups of trees are highly possible, as Roth (1953) and Hawksworth (1961) suggested for dwarfmistletoe on ponderosa pine. Hawksworth noticed a marked difference in percent of infection between groups of inoculated trees in a given area: 11 times greater in naturally infected stands than in previously uninfected stands. Differences in infection apparently were not associated with germination but with survival of the germinated seeds. Hawksworth suggests that possibly ecologic or climatic factors are also important in governing the distribution of dwarfmistletoe.

Microscopic observation of the process of penetration, as well as studies in which seeds were removed at regular intervals, showed that the length of time for infection to occur corresponded closely to the length of time necessary for the parasite to penetrate to the living tissues of the host. Results of these studies suggest that once the parasite reaches the living tissues of the branch it establishes a parasitic relationship with the host and is no longer dependent upon the seed for nutrient materials.

Death of the needle at the site of penetration and inhibition of the penetrating structure by the host was observed in red firs. In white firs needle dying was also observed, and possibly inhibition of the parasite also occurs. However, since no detailed investigations were made on this host, comments will be restricted to that which occurred on red firs.

Apparently red firs have a natural mechanism for resisting infection. During penetration an abscission zone often forms at the base of a needle and wound cork develops, inhibiting penetration by the parasite. Resistance to infection can be considered the result of a hypersensitive reaction, but this reaction does not appear to be one that involves host and parasite physiology. Instead, resistance is caused by the reaction of the host to physical pressures put upon the needle and branch by the holdfast and penetrating structure of the parasite. Further evidence that the host is reacting only to physical pressures is that penetration and infection in several cases occurred on the same branch where the needles were dying. Probably some seeds cause greater injury and stimulate more host reaction during penetration than others. Such resistance to infection undoubtedly is partially responsible for the rather low percent of infection on some firs and helps to keep the parasite in balance with the host.

Reports in the literature suggest that older branches of trees are more resistant to penetration and infection by dwarfmistletoe than younger branches (Weir 1916, Hawksworth 1961). The thick bark of older branches presumably is responsible for the increased resistance. In the present studies on firs, branch segments 1 to 5 years old showed no marked differences in susceptibility. Although thickness of bark was not critically determined, observations showed that on the 1- to 5-year-old segments

very little outer bark had formed. Somewhat older branches showed considerable outer bark formation. On older branches increased resistance may occur but was not tested in this investigation. In firs, therefore, a progressive increase in resistance to infection did not necessarily occur with an increase in branch age. More likely, resistance is related to the degree of outer bark formation. Probably susceptibility to infection remains nearly constant until a critical level of bark development occurs, at which time branches may become quite resistant.

INCUBATION PERIOD AND PRODUCTION OF SHOOTS, FLOWERS, AND FRUIT

The "incubation period" or period of latent disease development has been reported by several investigators and appears to vary considerably among the several species and forms of the parasite. Frequently included in these and other reports is information on the development of shoots, flowers, and fruit.

Heinricher (1915b) was the first investigator to observe in some detail the development of dwarfmistletoe from seed dispersal through shoot production. To study the parasite, he artificially inoculated potted junipers with *Arceuthobium oxycedri*. He found that seeds on hosts germinated, penetrated, and produced plants with shoot buds as early as 10 months after inoculation. The majority of the infections, however, produced shoot buds the year after germination. A few failed to produce buds until 2 years after germination. Later, Heinricher (1915c) briefly mentioned the length of time required for shoot development and flowering of plants resulting from his inoculations. He found that the earliest blooming of both male and female plants occurred in the third year after germination.

Results of the inoculations by Weir (1918) indicated that the time lapse between infection and appearance of shoots of some North American dwarfmistletoes is probably not over 2 or 3 years.

Hedgcock and Hunt (1917) reported that under greenhouse conditions plants of *Arceuthobium campylopodum* became established on several species of pines as early as 6 months after penetration, but did not mention the time required for the appearance of all plants that occurred. Some infections may have produced flower-bearing shoots within a year of penetration, since the investigators stated that in the second year, "All the trees inoculated successfully produced clusters of mistletoe plants in 1916, none of which produced mature fruits, apparently owing to lack of fertilization."

Gill (1954) has shown for *Arceuthobium vaginatum* in the Southwest that the shortest period between the time a seed alights on a stem and the first appearance of shoot buds, shoots with flowers, and shoots with mature fruit is 26, 33, and 48 months, respectively. Some plants, however, apparently do not produce shoots for 10 years or longer. Hawksworth (1961), also working with *A. vaginatum*, stated that the length of time a seed must remain in contact with the host to produce infection is probably at least 1 year. Also the time between noticeable swelling of the branch and the appearance of parasite shoots varied from

several months to 3 years. However, more than half the infections developed shoots within a year after branch swelling. Nearly all infections produced shoots 3 to 5 years after inoculation—about one-fourth the total in 3 years, an additional one-third in 4 years, and still another one-third in 5 years. A small percent produced shoots as early as 26 months and as late as 8 years. Plants first bore flowers in the fifth year, and mature fruits were produced 6 years after inoculations. Heavy fruiting began 7 years after inoculation.

Wagener (1962), studying *Arceuthobium campylopodum* on ponderosa and Jeffrey pines in California, reported that the period from seed placement to appearance of shoots was 3 to 6 years, 4 years being about average. He suggested that the inherent constitution of the individual host tree may influence the length of this period. In general, he found no correlation between the vigor of the host part and the time elapsing before shoot appearance.

Methods

The "incubation period" of dwarfmistletoes, as a review of the literature has shown, has various meanings. Most investigators have considered the period to extend from seed deposition to the appearance of parasite shoots. Others have considered it to extend from penetration to the appearance of shoots, and still others from infection to the appearance of shoots. To avoid confusion, we define "incubation period" in this study as "the period extending from seed deposition to the first macroscopic evidence of infection." In dwarfmistletoe on firs, branch swelling was the first indication of infection.

Infections resulting from inoculation of red fir and white fir at Latour and Pinecrest in 1958 were used for the study. At Pinecrest all infections were included, even those that appeared on untagged branches. At Latour only tagged infections were used.

Knowledge of the rate at which shoots, flowers, and fruit of dwarfmistletoe on firs are produced is necessary for understanding the life cycle of the parasite and for estimating its potential rate of buildup. To study these aspects of the parasite's growth, periodic observations were made on the dwarfmistletoe plants that occurred on the inoculated firs at Latour and Pinecrest. Records were kept from the time of initial seed deposition through the period of flowering and fruit production.

Results

Incubation period

At Latour, the incubation period on red firs varied from 2 to 4 years (fig. 22). The greatest number of infections appeared 2 years after seed deposition. About half as many infections appeared the third year and only about a fourth as many appeared the fourth year.

At Pinecrest the incubation period varied from 2 to 5 years. About half the infections appeared in the third year and about half in the fifth year. Only one infection appeared in the second year, and two in the fourth year. The infections that showed up in the third year occurred on main and secondary branches and were for the most part typical of

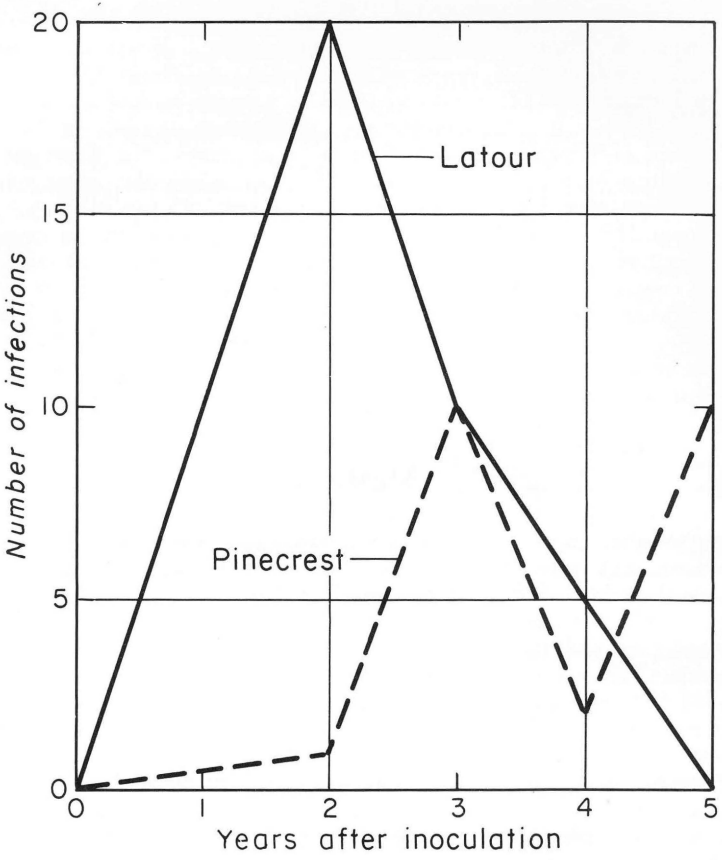

Figure 22.—**The incubation period of dwarfmistletoe on red fir.**

natural branch infections (fig. 23). On the other hand, all of the infections that appeared in the fifth year were tiny, "midget" infections (fig. 24). Almost all midget infections occurred on tertiary or quaternary branchlets.

No infections occurred on white firs at Latour. On white firs at Pinecrest only six infections occurred. Of these, four appeared 3 years after seed deposition and two 4 years after deposition. All but one—a midget that appeared in the fourth year on a tertiary branch—were typical infections on main or secondary branches.

Shoot, flower, and fruit production

At Latour, parasite shoots developed on red firs about 2 years after seed deposition from only about 25 percent of the infections observed, but none of the shoots bore flowers (fig. 25). The following year about 65 percent of the infections bore shoots, but less than 5 percent of these bore flowers. Four years after seed deposition about 80 percent of the infections bore shoots, about half of which bore flowers. One infection

F-513373

Figure 23.—Natural branch infection of dwarfmistletoe typical of the type occurring on white fir.

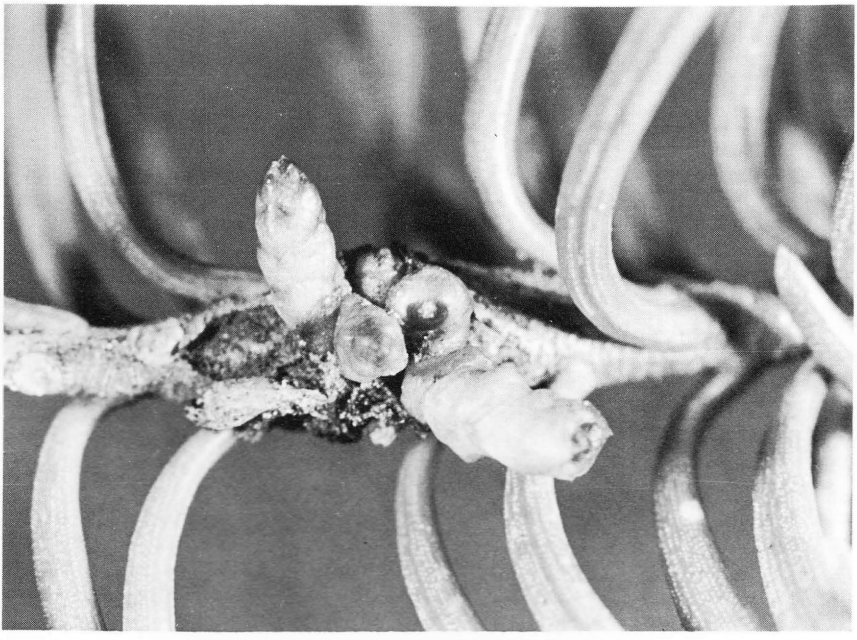

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Figure 24.—"Midget" infection of dwarfmistletoe on quaternary branch of red fir.

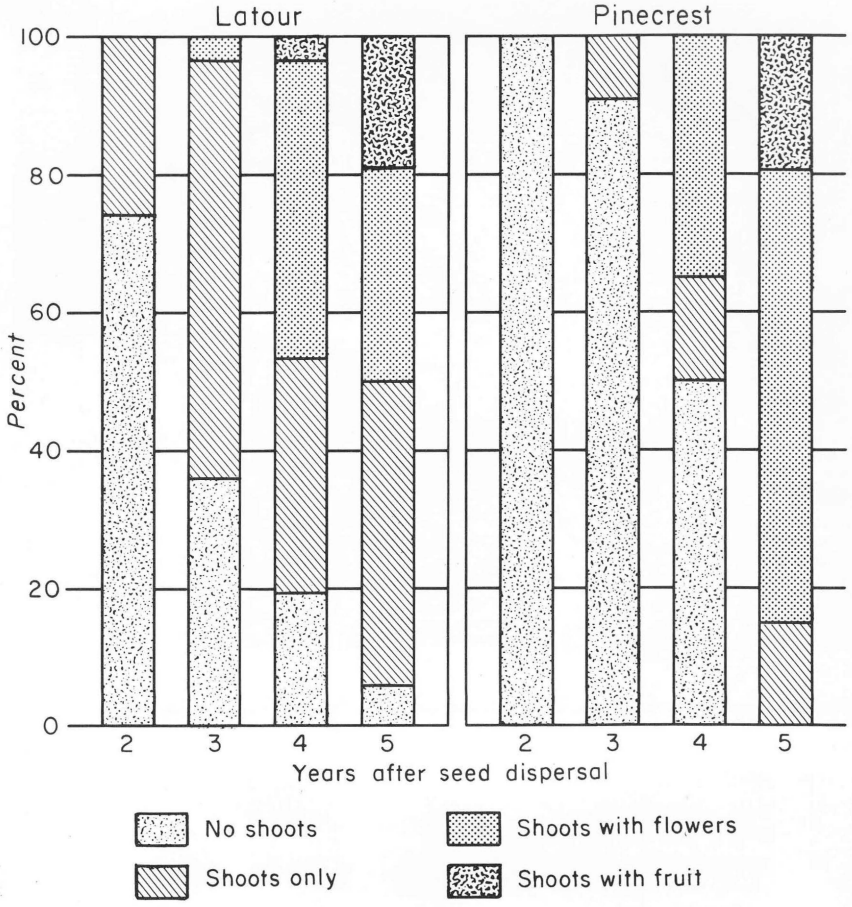

Figure 25.—**Occurrence of shoots, flowers, and fruit of dwarfmistletoe on red fir at yearly intervals after seed deposition.**

bore fruit. Five years after deposition about 5 percent of the infections still had failed to produce shoots, but about 20 percent of the infections with shoots bore fruit.

At Pinecrest no infections produced shoots the second year. Shoots were first observed the third year on about 10 percent of the infections, none of which bore flowers. The following year half the infections bore shoots, of which 60 percent bore flowers; none bore fruit. In the fifth year after seed discharge, all infections bore shoots. Of these, 65 percent bore flowers and 20 percent bore fruit.

On both red firs and white firs, shoots were produced only from the areas of swollen branch tissue. Once swelling of a branch occurred, parasite shoots were usually produced within 2 years. In only 5 percent of the cases did shoots fail to appear by the second year after swelling.

The larger, more vigorous infections occurred on the primary and secondary branches. Infections on these branches usually produced numerous shoots. Conversely, midget infections on tertiary and quaternary branches produced relatively few shoots.

In conclusion, the incubation period of infections at Latour generally differed from that at Pinecrest. At Latour symptom development occurred about a year earlier, and flowering and fruit production also occurred earlier.

For firs in general the incubation period varied from 2 to 5 years. Shoots with flowers were produced during the third to the fifth years. Few infections, however, produced fruit before the fifth year after seed deposition.

Discussion

Although the incubation period of dwarfmistletoe on red firs varied considerably among individual infections in a given area, the period was generally shorter for infections on primary and secondary branches than for infections on tertiary or quaternary branches. It is possible, as suggested by Heinricher (1915b) for *Arceuthobium oxycedri* on juniper, that vigor of the host branch influenced development of the parasite. Unfortunately, our studies did not compare the effects of primary and secondary branches with that of tertiary and quaternary branches. However, the capability of a branch to support an infection—rather than initial branch vigor—appeared to be the critical factor that determines the rate of parasite development. On the larger main and secondary branches, even those of lower vigor, a newly developing parasite probably obtains sufficient nutrients for initial growth and development without placing the host branch under severe nutrient stress. On the smaller tertiary and quaternary branches, however, it is likely that from the beginning of infection the parasite and host compete strongly for available nutrients. In these cases one might expect symptom development and growth of the parasite to be retarded.

Why the incubation period of most infections at Latour differed from that at Pinecrest was not determined. Such factors as tree vigor, age of branches inoculated, and exposure to light appeared more or less the same in both areas. Consequently, other factors, possibly climatic conditions or genetic or physiological differences in the host and parasite, influenced the length of the incubation period and development of the dwarfmistletoe infection.

For most infections, once swelling of a branch occurred, shoots were produced within a relatively short time. Presumably, therefore, initial branch swelling indicates the presence of an active, developing rootlike, nutrient-absorbing system capable of producing and supporting an aerial shoot system.

In damage to the host, infections on primary and secondary branches appear to be the most important. Infections on these branches become much larger and presumably block or utilize considerably more nutrients than infections on tertiary or quaternary branches. More important, the large infections produce the bulk of the seeds that are responsible for subsequent spread and intensification of the parasite.

Although relatively few infections occurred on white firs, the incubation period and time of shoot appearance following swelling appear not markedly different from that which occurs for dwarfmistletoe on red firs. The single "midget" infection on white fir also suggested that delayed development on tertiary and quaternary branches also occurs in this host species.

IMPLICATIONS FOR CONTROL

Sanitation cutting and pruning is a recognized approach to dwarfmistletoe control (Gill and Hawksworth 1954; Kimmey 1957; Parmeter and Scharpf 1963; Scharpf 1964). Usually this operation is carried out either in residual understory trees after logging of the infected merchantable stand or in young infected stands. The approach involves removing all trees from which infections cannot be pruned, and pruning all remaining infected trees.

Decision to apply this method usually must be based on the cost of control in relation to the degree of control obtained. Unless costs are sufficiently low and returns are sufficiently high, the investment in control is economically unsound.

To estimate the cost of control, it is necessary to consider the costs of initial sanitation and reworking, to remove escapes and latent infections. Initial cost usually can be calculated adequately, but the number and timing of reworkings depend on knowledge of the frequency of escapes or latent infections and the time necessary for escapes and latent infections to produce seed. Once escapes and latent infections have produced seed, a new generation of infections develops and additional reworkings become necessary, thus adding to the cost. The aim of the forest manager, therefore, should be to reduce the amount of latent and escape infections and to time reworkings to prevent subsequent fruiting of these infections.

One approach in reducing latent infections is to complete initial sanitation before the current season's mistletoe seeds have been discharged, thus eliminating a generation of infections that would otherwise appear after the sanitation operation. Our observations on red firs and white firs indicate that in California completion of sanitation before September is necessary to prevent deposition of seed.

Once initial sanitation is completed, the appearance and fruiting of latent and overlooked infections will govern the number and timing of reworkings. Assuming that initial sanitation is completed before September and that all infections with shoots are removed, the following types of infections may be expected to appear in subsequent years: (a) Infections overlooked because of lack of shoots and (b) infections arising from seeds deposited prior to control.

Observations and results of the present study indicate that infections without shoots and those with only shoot buds ordinarily require a minimum of 1 year to produce shoots, 1 year to produce flowers, and 1 year to produce fruit. Thus, while a rare fruit may be produced in 2 years, the minimum period required for appreciable fruiting is 3 years. These data suggest that, if initial sanitation removes all infections with shoots, reworking before September in the third year after initial control will prevent further seed deposition. If all infections with shoots are again removed at this time, a second reworking before September in the sixth year should remove most of the infections from the stand. The result should be 7 years without seed deposition, which is considered sufficient time to allow practically all latent infections to appear.

Experience with sanitation as a control of dwarfmistletoe in other host-parasite associations (Gill and Hawksworth 1954; Wagener 1965) indicates that complete eradication is a practical impossibility. The procedure recommended here, however, should reduce the population to a very low level. The effectiveness of sanitation as an economical control method depends on the rate at which a mistletoe population can increase from this low level.

The present study does not include observations on population increase, but information on generation time and percentage infection should aid in the prediction of future population levels and in the interpretation of existing populations. Seeding experiments reported in this study showed that the minimum generation time of dwarfmistletoe on fir was, for practical purposes, 5 to 6 years, and in three out of four instances percent of infection from deposited seeds was low. Thus, observations on population levels should be interpreted on the basis of a 5- to 6-year life cycle and in all likelihood a rather low rate of initial buildup. Results of the present investigations indicate, therefore, that it takes many years and several generations of dwarfmistletoe before low levels of infection reach damaging proportions in firs.

LITERATURE CITED

California Forest Pest Control Action Council.
1961. forest pest conditions in california. Official report, Calif. Forest Pest Control Action Council, Sacramento, Calif.

Cannon, W. A.
1904. observations on the germination of *Phoradendron villosum* and *P. californicum*. Torrey Bot. Club. Bul. 31: 435–443.

Cohen, Leon I.
1963. studies on the ontogeny of the dwarfmistletoes, *Arcethobium*. ii. homology of the endophytic system. Amer. Jour. Bot. 50: 409–417.

Dowding, Eleanor S.
1929. the vegetation of alberta. iii. the sandhill areas of central alberta with particular reference to the ecology of *Arceuthobium americanum* nutt. Jour. Ecol. 17: 82–105.

Gill, Lake S.
1935. *Arceuthobium* in the united states. Conn. Acad. Arts and Sci. Trans. 32: 111–245.

———
1954. dwarf mistletoe of ponderosa pine in the southwest. U.S. Forest Serv. Rocky Mountain Forest and Range Expt. Sta. Paper 14, 9 pp., illus.

——— and Hawksworth, F. G.
1954. dwarfmistletoe control in southwestern ponderosa pine forests under management. Jour. Forestry 52: 347–353.

Hawksworth, Frank G.
1954. observations on the age of lodgepole pine tissues susceptible to infection by *Arceuthobium americanum*. Phytopath. 44: 552.

———
1961. dwarfmistletoe of ponderosa pine in the southwest. U.S. Dept. Agr. Tech. Bul. 1246, 112 pp., illus.

Hedgcock, George G., and Hunt, N. R.
1917. notes on *Razoumofskya campylopodum*. Phytopath. 7: 315–316.

Heinricher, E.
1915a. beiträge zur biologie der zwergmistel, *Arceuthobium oxycedri*, besonders zur kenntnis des anatomischen baues und der mechanik ihrer explosiven beeren. Akad. der Wiss. Wien, Math.-Naturw. 124(1): 181-230.

———
1915b. die keimung und entwicklungsgeschichte der wacholdermistel, *Arceuthobium oxycedri*, auf grund durchgeführter kulturen geschildert. Sitzungsber. Akad. Wiss. Wien, Abt. I, 124(1): 319–352.

———
1915c. über bau und biologie der blüten von *Arceuthobium oxycedri* (dc.) mb. Akad. der Wiss. Wien, Math.-Naturw. Kl. Sitzber. Abt. 124(1): 481–504, illus.

Kimmey, J. W.
1957. dwarfmistletoes of california and their control. Calif. Forest and Range Expt. Sta. Tech. Paper 19, 12 pp., illus.

KUIJT, JOB.
1955. DWARF MISTLETOES. Botan. Rev. 21: 569–627, illus.

———
1960. MORPHOLOGICAL ASPECTS OF PARASITISM IN THE DWARFMISTLETOES (*Arceuthobium*). Univ. Calif. Pub. in Bot. 30: 337–436.

MACDOUGAL, D. T.
1899. SEED DISSEMINATION AND DISTRIBUTION OF *Razoumofskya robusta* (ENGELM.) KUNTZE. Minn. Bot. Stud. S. 2 pt. 2, 169–173, illus.

MCLUCKIE, J.
1923. STUDIES IN PARASITISM: A CONTRIBUTION TO THE PHYSIOLOGY OF THE LORANTHACEAE OF NEW SOUTH WALES. Bot. Gaz. 75: 333–369, illus.

MENZIES, B. P.
1954. SEEDLING DEVELOPMENT AND HAUSTORIAL SYSTEM OF *Loranthus micranthus* HOOK. F. Phytomorph. 4: 397–409.

PARMETER, J. R., JR., and SCHARPF, R. F.
1963. DWARFMISTLETOE ON RED FIR AND WHITE FIR IN CALIFORNIA. Jour. FORESTRY 61: 371–374, illus.

———, ———, and HOOD, J. R.
1960. HOST SPECIALIZATION OF DWARFMISTLETOE ON RED AND WHITE FIR IN CALIFORNIA. Phytopath. 50: 650. (Abstract.)

PEIRCE, G. J.
1905. THE DISSEMINATION AND GERMINATION OF *Arceuthobium occidentale* ENG. Ann. Bot. 19: 99–113.

ROTH, L. F.
1953. PINE DWARFMISTLETOE ON THE PRINGLE FALLS EXPERIMENTAL FOREST. U.S. Forest Serv. Pacific Northwest Forest and Range Expt. Sta. Res. Note 91, 3 pp.

———
1959. NATURAL EMPLACEMENT OF DWARFMISTLETOE SEED ON PONDEROSA PINE. Forest Sci. 5: 365–369, illus.

SCHARPF, ROBERT F.
1964. DWARFMISTLETOE ON TRUE FIRS IN CALIFORNIA. U.S. Dept. Agr. Forest Pest Leaflet 89, 7 pp., illus.

THODAY, D.
1951. THE HAUSTORIAL SYSTEM OF *Viscum album* L. Jour. Expt. Bot. 2: 1–19.

WAGENER, W. W.
1961. THE INFLUENCE OF LIGHT ON ESTABLISHMENT AND GROWTH OF DWARFMISTLETOE ON PONDEROSA AND JEFFREY PINES. U.S. Forest Serv. Pacific Southwest Forest and Range Expt. Sta. Res. Note 181, 5 pp.

———
1962. DWARFMISTLETOE INCUBATION PERIOD ON PONDEROSA AND JEFFREY PINES IN CALIFORNIA. Forest Sci. 8: 16–20.

———
1965. DWARFMISTLETOE REMOVAL AND REINVASION IN JEFFREY AND PONDEROSA PINES, NORTHEASTERN CALIFORNIA. U.S. Forest Serv. Pacific Southwest Forest and Range Expt. Sta. Res. Note 73, 8 pp.

WEIR, J. R.
1916. MISTLETOE INJURY TO CONIFERS IN THE NORTHWEST. U.S. Dept. Agr. Bul. 360, 39 pp., illus.

———
1918. EXPERIMENTAL INVESTIGATIONS ON THE GENUS *Razoumofskya*. Bot. Gaz. 66(1): 1–31.

YORK, H. H.
1909. THE ANATOMY AND SOME OF THE BIOLOGICAL ASPECTS OF THE "AMERICAN MISTLETOE" *Phoradendron flavescens* (PURSH.) NUTT. Texas Univ. Bul. 20, Sci. Ser. 13, 31 pp., illus.

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